Abstracts

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Abstracts for the oral and poster presentations are provided in this special on-line supplement.
Pharmacological Inhibition of PCSK9 in Hyperlipidemic Mice Significantly Reduces Serum LDL-C While Increasing Hepatic Low-Density Lipoprotein Receptor Protein Abundance

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a member of the proprotein convertase family of proteases that has been previously demonstrated to promote the degradation of the low density lipoprotein receptor (LDLr) through an undefined mechanism. Mutational analysis in humans has demonstrated that genetic polymorphisms which inactivate PCSK9 produce dramatic reductions in serum LDL-C and further, appear to reduce the risk of coronary artery disease (CAD). These encouraging epidemiological findings have been corroborated using PCSK9 knockout mice, which exhibit the same phenotypic profile (i.e. decreased LDL-C and increased hepatic LDLr). For this reason, we have developed second generation antisense oligonucleotide inhibitors (ASOs) which target both human and mouse forms of PCSK9 mRNA to define their hypolipidemic effects both in vitro and in vivo. Due to their optimal pharmacokinetic/pharmacodynamic properties, 2-MOE-modified ASOs have been extensively exploited to inhibit a broad range of therapeutically attractive liver gene targets such as apoE, Lp(a) and ACAT2. Administration of the PCSK9 ASO (ISIS 394814) to high fat fed mice for 6 weeks (i.p., 100mg/kg/wk) resulted in reductions in both total cholesterol and LDL-C, 53% and 38%, respectively. In addition, hepatic RNA and protein analysis revealed that ISIS 394814 reduced PCSK9 mRNA expression by 92% while increasing LDLr protein levels greater than 2-fold relative to controls. The magnitude of these effects is consistent with results previously reported in PCSK9 knockout mice. Based on these data, additional studies are in progress in LDLr deficient and additional mouse models to further demonstrate the specificity and pharmacological efficacy of this drug. These promising in vivo results suggest that inhibiting PCSK9 may indeed represent a novel therapeutic approach for reducing LDL-C in man.

Impact of Apolipoprotein M Expression on Nascent Pre-β HDL Formation by ATP Binding Cassette Transporter A1 (ABCA1)


ApoM is a novel apolipoprotein that mainly associates with high density lipoproteins (HDLs) in plasma and has been reported to play a role in pre-beta (preb) HDL formation. We investigated the role of apoM expression on the initial steps of nascent preb HDL particle assembly by ABCA1 using Hek293 cells. Neither control nor ABCA1 expressing Hek293 cells expressed detectable apoM mRNA or protein based on real time PCR and Western blot analysis, respectively. Control and ABCA1-expressing cells were transfected with apoM-C-FLAG and radiolabeled with 35S-Met/Cys prior to immunoprecipitation of apoM from medium and cell lysates. Results showed that only 1% of the total cellular apoM was secreted both in the absence or presence of ABCA1 expression. Immunofluorescence with anti-FLAG antibody demonstrated that apoM protein was located primarily at intracellular sites and not at the plasma membrane of transfected cells. To investigate the role of apoM in nascent preb HDL formation, ABCA1-expressing or control cells, transfected with empty vector, apoM WT or apoM C-FLAG, were incubated with 125I-free apoA-I for 24 hours. Conditioned media were harvested and fractionated by FPLC to observe HDL particle size distribution as monitored by elution of 125I-apo. Preb HDL particles were formed in the absence of apoM expression (WT or C-FLAG); however, apoM expression promoted formation of larger-sized nascent preb HDL. Immunoprecipitation of the different sized FPLC-isolated preb HDL with anti-apoA-I antibody followed by apoM Western blot analysis of pellet and supernatant showed that very little secreted apoM associated with preb HDL particles. Our results suggest that apoM is poorly secreted by Hek293 cells, and is not required for preb HDL assembly by ABCA1, and interacts poorly with secreted nascent preb HDL. However, apoM expression results in the appearance of larger preb HDL particles in media. We propose that apoM may function catalytically at an intracellular site to transfer lipid onto preb HDL during or after their formation by ABCA1.

Intestinal Cholesterol Absorption Is Required for the LXR Agonist to Increase Plasma HDL Cholesterol in Mice

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Genetic manipulation has established the liver as the major source of HDL in normal mice. However, there is about 30% of plasma HDL contributed to the small intestine. It has been reported that liver X receptor (LXR) agonist GW3965 raises plasma HDL cholesterol levels in both wildtype and L1-KO mice although hepatic ABCG5/G8 mRNA levels were increased in both wildtype and L1-KO mice whereas hepatic ABCG8 mRNA levels were reduced in both wildtype and L1-KO mice. However, hepatic ABCG8 mRNA levels were reduced in both wildtype and L1-KO mice in which a much greater fecal cholesterol level but this effect was almost abolished in the L1-KO mice in which a much greater fecal cholesterol level was observed. Therefore, the liver X receptor (LXR) agonist fraction (12-LO) (L1-KO mice), a gene that is essential for intestinal cholesterol absorption, were treated with the LXR agonist T0901317 at 25 mg/kg BW/day for 7 days. As expected, T0901317 treated wildtype mice showed a dramatic increase in the plasma HDL cholesterol level but this effect was almost abolished in the L1-KO mice in which a much greater fecal cholesterol excretion was observed instead. The intestinal ABCA1 mRNA level was about 4-fold lower in the untreated L1-KO versus wildtype mice, and increased 4.4-fold and 7.6-fold in the T0901317-treated wildtype and L1-KO mice, respectively. Hepatic ABCA1 failed to respond to T0901317 in both wildtype and L1-KO mice although hepatic ABCG5/6 mRNA levels were higher in the T0901317-treated versus untreated animals. In conclusion, intestinal cholesterol absorption is required for LXR agonist to increase plasma HDL cholesterol in mice.

HDL Transport Through Endothelial Cells Is Mediated by ABCG1 and SR-B1

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High density lipoproteins (HDL) and their main protein constituent, apolipoprotein A-I (apoA-I), exert potentially anti-atherogenic properties within the arterial wall. However, it is not clear how HDL can be transported through the apical to the basolateral compartment as many studies have shown that HDL cannot pass through the tight junctions between endothelial cells. Endothelial cells bound and cell associate both 125I-HDL and 125I- apoA-I in a saturable and non-saturable manner. Binding and cell association of HDL was only competed by excess HDL not by apoA-I and albumin. In contrast binding and cell association of apoA-I is competed by excess apoA-I and HDL and not by albumin. Biotinylaton experiments showed that endothelial cells internalize labeled HDL and only apoA-I. Western blot analysis of media, in a well system, the cells transported a fraction of 125I-HDL from the apical to the basolateral compartment as an intact protein in a compatible and temperature sensitive manner. Moreover, RNA interference to modulate ATP binding cassette G1 (ABCG1) and SR-BI resulted in reduced HDL cell uptake, binding, internalization and transport. We conclude that endothelial cells transport HDL in an ABCG1 and/or SR-BI dependent process.

Docosahexaenoic Acid Impairs the Formation of Fully Lipidated VLDL by Oxidative Modification, Aggregation, and Degradation of the Precursor Particles

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Background: One mechanism of the lipid-lowering effects of n-3 fatty acids including docosahexaenoic acid (DHA) is reduced hepatic VLDL secretion by promoting intracellular apolipoprotein B (apoB) degradation. We reported that this is a non-proteasomal post-ER process, activated by intracellular conversion of DHA to lipid peroxides. DHA-treatment of hepatocytes also resulted in the intracellular formation of malondialdehyde-modified high molecular weight apoB aggregates. Objective: To determine the stage in the VLDL secretory pathway where oxidative modification and degradation of apoB occurs. Results: In pulse-chase studies, initial stages (up to 30 min of chase), C8bL of lipoprotein assembly was significantly different between oleic acid (OA) and DHA treated cells, with similar luminal quantities of total apoB, pre-VLDL and VLDL. At C45, compared to OA, a decrease in luminal apoB was observed in DHA-treated cells, but in contrast to OA, this apoB was not recovered in the medium. Interestingly, the C45 time point at which DHA coincided with accumulation of apoB aggregates partially accounted for the loss of apoB from the medium. apoB from Golgi-specific microsomes. Co-administration of deferoxamine (DFX), an iron-chelator that prevents lipid peroxidation, caused DHA-treated cells to assume an OA-like profile, accumulating luminal VLDL apoB that was secreted. Using an antibody for light-chain 3 (LC3), a specific autophagosomal marker, C8bL apoB aggregates were shown to be enriched in cells treated with DHA compared to OA or DHA + DFX. Conclusions: (1) DHA allows step 1 (pre-VLDL particle formation) and their transport to the Golgi (2) Oxidative damage of the pre-VLDL apoB and/or its association with peroxidized lipids in the Golgi results in reduced luminal formation of secretion-competent VLDL particles (3) Aggregated apoB is co-localized with autophagosomes.

12-Lipoxygenase Deficiency Inhibits Angiotensin II-induced Abdominal Aortic Aneurysm Rupture and Incidence in Apolipoprotein E-deficient Mice

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Objective: Arachidonic acid is metabolized through the cyclooxygenase-1 and -2 (COX-1 and -2), 5-lipoxygenase (5-L0) and 12/15-lipoxygenase (12-L0) pathways which produce a host of biologically active fatty acid derivates that affect cardiovascular disease. We have recently demonstrated that selective pharmacological inhibition of the COX-2 pathway markedly attenuated Angiotensin II (AngII)-induced abdominal aortic aneurysm (AAA) formation. Interestingly, SC-L0 deficiency also attenuated AAA formation in an experimental animal model of AAAs. Genetic or pharmacological inhibition of one of these pathways may result in the shunting of arachidonic or linoleic acid down the 12-L0 pathway. Therefore, we sought to determine the effect of 12-L0 deficiency on AngII-induced AAA formation. Methods: Age matched male C57BL/6 apoE-deficient (12-L0 -/-) and C57BL/6 12-L0 deficient apoE-/- mice were infused with...
Reoxygenation Leads to Dissociation of Histidine Deacetylase 7 from Hypoxia-inducible Factor-1a

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 Interruption of cerebral blood flow causes hypoxia leading to the death of neurons and glia. Hypoxia-inducible factor-1a (HIF-1a) plays an important role in cell survival under oxygen deprivation by regulating the transcription of genes involved in glucose metabolism, cell proliferation, and angiogenesis. However, expression of genes targeted by HIF-1a include vascular endothelial growth factor (VEGF), erythropoietin, and glucose transporter-1, which play a role in improved cell survival under hypoxia. When oxygenation is normal, HIF-1a is rapidly degraded through the ubiquitin-proteasome pathway, which is triggered by the oxygen-dependent hydroxylase enzyme prolyl hydroxylase (PHD). Hydroxylated proline residues are recognized by von-Hippel-Lindau tumor suppressor protein (pVHL), a component of an E3 ubiquitin ligase complex (pVHL, Elongin B, and Elongin C). The E3 ubiquitin ligase complex promotes ubiquitination of HIF-1 leading to degradation of HIF-1a. Under hypoxia, these hydroxylated proline residues are not recognized and the oxygen-dependent degradation system is repressed. Previously, we identified histidine deacetylase 7 (HDAC7) as a binding protein of HIF-1a using yeast two-hybrid system. HDAC7 was also shown to increase HIF-1a transcriptional activity under hypoxia. In this study, we investigated roles of HDAC7 in hypoxia-induced angiogenesis. Our results showed that degradation of HIF-1a was correlated with translocation of HDAC7 from the nucleus to the cytoplasm upon reoxygenation. Using a mutant of HIF-1a (Pro402A/Pro564A), we also found that the stabilized HIF-1a mutant localized in the nucleus under re-oxygenation whereas HDAC7 was exported to the cytoplasm. The amino acids substitution mutations of nuclear export sequences (NES) in HDAC7 (NES mut.) blocked translocation of HDAC7 to the cytoplasm and stabilized HIF-1a in the nucleus upon re-oxygenation. Moreover NES mut HDAC7 bound HIF-1a upon re-oxygenation. Taken together, these results suggest that the dissociation of HDAC7 from HIF-1a and translocation of HDAC7 to the cytoplasm may lead to degradation of HIF-1a upon re-oxygenation.

10 Role of Autotaxin, a Plasma Lysophospholipase D, in Hemostasis and Thrombosis

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Lysophosphatic acid (LPA) is a bioactive lipid mediator produced by platelets and found in plasma and atherosclerotic plaques. LPA stimulates platelets, leukocytes, endothelial cells, and smooth muscle cells to regulate cell growth, differentiation, survival, motility, and contractility. LPA is thus poised to serve as a key regulator of vascular cell function. LPA is generated in large part by the secretory lysophospholipase D autotaxin (ATX, ep2s). Mice with only one ATX gene (ep2s/-) have plasma LPA levels that are 50% of wild type mice. ATX deficiency in mice (ep2s/-) results in embryonic lethality due to vascular defects, implicating ATX and potentially LPA in vascular development. We observed excessive bleeding in three founder lines of transgenic FVB mice globally overexpressing ATX (ATX-Tg). To characterize the bleeding defect further, tail vein bleeding times were performed. The mean bleeding time in control FVB mice was 3 ± 2.7 min (n = 6), whereas none of the ATX-Tg mice (n = 10) stopped bleeding within 10 min (p < 0.001). Platelet counts were similar in control (828 ± 184 x 10^7/mm^3) and ATX-TG (808 ± 156 x 10^7/mm^3), and platelets from control and ATX-TG mice displayed similar levels of platelet membrane glycoprotein (GpIIb, GpIIa, GpIIIa) as measured by flow cytometry. Upon stimulation by different agonists (ADP, collagen, thrombin), platelets from ATX-TG mice also expressed P-selectin and bound fibrinogen as did control platelets. No differences in shear-induced platelet aggregation in whole blood from control and ATX-TG mice were observed (surface coverage 10.25 ± 1.73% and 9.21 ± 1.6%, respectively, p = 0.595). Thus, ex vivo studies were not able to recapitulate a platelet function defect. Additionally, clotting times were normal in the ATX-TG mice. To determine if thrombosis was altered in the ATX-TG mice, mice were studied in the ferric chloride-induced carotid artery thrombosis model. In control mice, thrombus formation occurred the vessel in 10 ± 1.4 min (n = 3), whereas none of the ATX-TG mice (n = 3) showed occlusion throughout 30 min (p < 0.001). In summary, our results suggest that ATX, and potentially LPA, regulate hemostasis and thrombosis through effects on vascular function.

11 Variable Growth Rates and Diameter-Dependent Expression of Vascular Endothelial Growth Factor Receptors in Experimental Abdominal Aortic Aneurysms

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Purpose: Transmural inflammatory and adventitial neovascularization are important pathophysiologic correlates of both human and experimental abdominal aortic aneurysm disease progression. We hypothesized that adventitial VEGF receptor expression would be increased in diseased compared to non-diseased segments of abdominal aortic aneurysms.

Methods: 100 E1 mice deficient in a C57/Bl6 background were infused with ATX, and potentially LPA, regulate hemostasis and thrombosis through effects on vascular function.
intravenously (10μg/mouse) was performed on select mice (n = 3) once AAA diameter reached at least 175% of baseline aortic diameter. Results: All AAA mice identified were suprarenal and exhibited a variable growth rate. By postoperative day 12, 45% of the mice demonstrated a large AAA (defined as 175% or greater lumen diameter dilation compared to normal) with transabdominal ultrasound. We visualized large AAs in 82% of the mice by postoperative day 24, and 95% of the mice by postoperative day 30 with ultrasound. In vivo and ex vivo fluorescence imaging of VEGF receptors with scVEGF-Cys5 in select mice demonstrate increased signal in the larger AAA (24% dilated) compared to the smaller AAA (183% dilated).

Conclusion: We have demonstrated a variable growth pattern and size dependent enhancement in VEGF-2 expression in a mouse model of AAA disease. VEGF receptor may prove useful as a clinical marker of AAA progression.

14 A Novel Translational Pathway Represses Vascular Endothelial Growth Factor Expression and Angiogenic Activity by Activated Monocytes

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Post-transcriptional regulation of gene expression in macrophages is a principal mechanism limiting inflammation. Early pro-inflammatory actions of the cytokine interferon-γ (IFN-γ) later switch to anti-inflammatory, contributing to inflammation resolution. In activated monocytes, IFN-γ elicits formation of the IFN-γ-activated inhibitor of translation (GAIT) complex that inhibits translation of ceruloplasmin, an acute phase inflammatory protein. Bioinformatic and ribonucleoprotein-microarray analyses reveal multiple transcripts as potential targets of GAIT-mediated translational silencing. Our identification of the angiogenic factor VEGF as a candidate target was particularly significant because it is a potent angiogenic factor mediating inflammation and tumor angiogenesis. VEGF translation tends to be suppressed in macrophages in tumour microenvironments and in chronic inflammatory conditions such as atherosclerosis; however, no negative regulatory mechanisms are known. We show that VEGF mRNA in activated monocytes is silenced by GAIT complex-binding to a defined 2'TRNA RNA element. IFN-γ increases VEGF translation repressor titres which delays silencing of VEGF protein synthesis via negative regulation of macrophage angiogenic activity as shown by endothelial cell proliferation and tube-formation assays. Our results are the first to show negative regulation of VEGF expression under inflammatory conditions. GAIT-mediated translation repression of VEGF, and other inflammatory mRNAs, may provide a new anti-angiogenic role response to inflammatory stimuli and contributes to the resolution of chronic inflammation.

15 Atoxl as a Novel Copper-dependent Transcription Factor for Cell Proliferation: Role in Neovascularization

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Copper plays a fundamental role in regulating cell proliferation involved in angiogenesis. Recently, we found that antioxidant-1 (Atoxl), previously appreciated as a copper chaperone, functions as a novel copper dependent transcription factor that mediates copper-induced cell proliferation. We performed present study to test the hypothesis that Atoxl play an important role in postnatal neovascularization. Using mouse models of ischemic hindlimb model, we found that neovascular formation in the ischemic hindlimb was significantly impaired in Atoxl−/− mice as compared with WT (35.9±6.7% decrease) as evaluated by laser Doppler blood flow. Capillary density in ischemic hindlimb at 2 days after ischemia was markedly reduced in Atoxl−/− mice as compared with WT mice (27.8±4.7% decrease). In addition, supernatant of Atoxl−/− macrophages which plays a critical role in angiogenesis was significantly decreased in ischemic hindlimb of Atoxl−/− mice as examined by lucigenin assay (33.5±2.5% decrease). Interestingly, the numbers of CD34/CD31− and c-kit/CD31− double positive endothelial progenitor cells (EPCs) derived from bone marrow and peripheral blood were markedly decreased in Atoxl−/− mice after ischemia as compare to WT (37.5−22.4% decrease, respectively) as analyzed by FACS. To gain insight into the molecular mechanism, we performed EMSA and CHIP assays and found that Atoxl binds to the promoter region of cyclin D1 in a copper dependent manner. Moreover, copper-induced increase in cell proliferation and protein levels of cyclin D1 is markedly inhibited in Atoxl deficient mouse fibroblasts and by knockdown of Atoxl using siRNA. Taken together, we provide the first evidence that Atoxl functions as a copper dependent transcription factor for cyclin D1 and thus stimulates cell proliferation, thereby promoting neovascularization induced by tissue ischemia.

16 Candidate Susceptibility Loci for Vascular Remodeling Identified Through a Genome-wide Association Study of In-stent Restenosis

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Introduction: Risk-conferring genes responsible for complex diseases are characterized by variable expressivity, low penetrance, epistasis and locus heterogeneity, making the analysis of complex genetic traits challenging. For complex cardiovascular diseases, candidate gene approaches have achieved limited success, making genome-wide association study (GWAS) designs appealing. Molecular and genetic studies suggest that in-stent restenosis (ISR) is primarily an inflammatory and proliferative disease, with distinct roles for cell cycle proteins, growth factors, and inflammatory cytokines.Methods: To investigate the genetic basis of ISR, we designed a case control GWAS on a dataset of ~116,000 single nucleotide polymorphisms (SNPs) assayed in 407 patients (150 cases, 257 controls). We undertook a haplotype analysis of regions highlighted by the presence of two or more SNPs within 250 kb of one another and with p<0.001 (unadjusted) in univariate tests of allelic association. Haplotypes were defined in the regions and tested for association with ISR, using a Bonferroni correction for all haplomarker and tumor angiogenesis. VEGF, a functional result, tested: Results: We identified three of the five loci, each harboring at least one genetic susceptibility haplotype, and we found that Atoxl, a hypothetical amino acid sequence first identified when examining the time to development of ISR as a quantitative trait in a Cox regression model and demonstrated a significant effect of allele dose for each of the eight regions identified (p<0.001038 to 0.035, after Bonferroni correction), suggesting that allele copy number contributes to ISR. In addition, we performed a MasterQ assay in a selected group of 106 patients to call genotypes and identified some of the same genes and eP491 and 5718. The additional
regions demonstrate significant allele dose effect as well (p<0.003). Conclusions: Haplotype analysis of GWAS using SNP markers is a useful approach to identify candidate genes for complex vascular diseases. These areas of association with ISR warrant further investigation.

17 Ambient Particulate Pollutants in the Ultralate Range Promote Atherosclerosis and Systemic Oxidative Stress

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Background - Air pollution has been associated with significant adverse health effects leading to increased cardiovascular morbidity and mortality. Cumulative evidence supports the hypothesis that the particle size of particulate matter is closely related to adverse health effects. EPA is currently classifying particles with an aerodynamic diameter of < 2.5 μm (PM2.5), there is increasing evidence that the smaller particulate components shed into polluted urban air from vehicular emissions may even be more dangerous due to their high content of pro-oxidative chemicals. Methods and Results - To test our hypothesis that the smaller the particles, the larger the cardiovascular effect, we used particle concentrator technology to compare the atherogenic effects of ambient particles with aerodynamic diameter < 0.18 μm (ultrafines) vs. fine particles < 2.5 μm (PM2.5) in downtown Los Angeles. Two experimental protocols were used. In the first, 6-week-old male C5BL/6J apoe null mice were placed on a chow diet and exposed to concentrated ambient PM (CAPs) for a combined total of 75 hours over a period of 40 days; while in the second, 2-month-old male apoe null mice were fed a high fat diet (HFD) and exposed to CAPs for a combined total of 120 hours over a period of 58 days. Control groups included mice exposed to filtered air (FA) or left non-exposed (NE). Chow-fed mice exposed to concentrated ultrafines developed 25%, 55% and 91% greater atherosclerotic lesions than animals exposed to PM2.5, FA or NE mice, respectively (n=14–17/group, p<0.05). Exposure to ultrafine particles resulted in an inhibition of the antinflammatory capacity of plasma high density lipoproteins and increased systemic oxidative stress markers as evidenced by a significant (i) increase in liver lipid peroxidation, (ii) upregulation of Nrf2 and Nrf2-related phase-2 response antigens (e.g. catalase, superoxide dismutase, NQO-1). HFD-fed apoe null mice exposed to concentrated ultrafines also exhibited evidence of increased systemic oxidative stress but not of greater atherosclerosis. Conclusions - Ultrafine particles promote the proatherogenic effects of ambient PM and constitute a significant cardiovascular risk factor that may need to be considered in air quality regulation, similar to PM2.5.

18 Induction of Apoptosis in Established Atherosclerotic Lesions Promotes Inflammation and Monocyte Recruitment in Apoe-/– Mice

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Introduction: The impact of apoptosis on atherosclerosis progression may be deleterious as apoptotic cell clearance appears to be defective in atherosclerotic plaques. We evaluated the impact of ApoE deficiency on the induction of apoptosis and monocyte recruitment in a new genetically-modified mouse model. Methods: Mice expressing the Diphteria Toxin Receptor (DTR) under the control of the Cd1c promoter were crossed with Apoe-/– mice. Induction of apoptosis in Cd1c– lesional cells was achieved by injection of Diphteria Toxin (DT, 4ng/g). Apoe–/– and DTR Apoe–/– mice were fed a Western diet for 8 weeks and divided into 3 groups: ApoE–/– + DT (control), DTR Apoe–/– + PBS (control) and DTR Apoe–/– + DT. Two days after injection, chemokines and monocyte marker expression was evaluated in the aorta, while apoptosis and newly recruited macrophage detection were assessed in aortic root lesion. Results: Increased TUNEL staining in the aortic tissue of DTR Apoe–/– + DT mice as compared to control mice (p<0.05) was associated with increased mRNA expression for the small inducible chemokines MCP-1, MIP-1α, MIP-1β and MIP-2 (p<0.05 vs controls). Concomitantly, mRNA expression of monocyte markers (CD11b, CD11c, CD68) was significantly increased in the aorta of DTR Apoe–/– + DT mice as compared to controls. Moreover, analysis of aortic root lesions revealed the presence of newly recruited macrophages in areas of apoptotic cell accumulation, which was indicative of monocyte recruitment. Monocyte tracing using fluorescent beads confirmed that apoptotic cell death promoted recruitment of circulating monocytes in the aorta of DTR Apoe–/– + DT mice. Conclusions: These data suggest that apoptosis associated with impaired apoptotic cell clearance may promote inflammation and recruitment of monocytes in established atherosclerotic lesions.

19 Distinctive Expression of Chemokines and Transforming Growth Factor-β Signaling in Human Arterial Endothelium During Atherosclerosis

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Objectives: Knowledge about the in vivo role of endothelium in chronic human atherosclerosis has mostly been derived by insights from mouse models. Therefore, we set out to establish by microarray analyses the gene expression profiles of endothelium from human large arteries, isolated by Laser Microbeam Microdissection, having focal atherosclerosis of the early or the advanced stage. Within individual arteries the endothelial transcriptomes of initial (N=7) – and advanced (N=6) lesions were compared pairwise to the unaffected sites, thus limiting genetic and environmental confounders. Results: Specific endothelial signature gene sets with changed expression levels in either (N=7) – or advanced atherosclerosis (N=403), relative to their paired plaque-free controls, were identified (paired t-test: FDR-corrected p<0.05). Gene Set Enrichment Analysis identified distinct sets of chemokines and differential enrichments (p<0.05) of NF-kappa B-, p53- and TGF-β-related genes in advanced plaques, compared with initial plaques. Immunohistochemistry on a separate set of human arteries with focal atherosclerosis of the early- (N=6) or the advanced-stage (N=5) – validated the direction and value of corresponding changes in endothelial protein expression between early (focal) and advanced stages of atherosclerosis and versus their plaque-free controls. Conclusions: The functional involvement of TGFbeta-signalizing in directing its downstream gene repertoire was substantiated by a consistent picture of activated TGFβ SMAD2 in early, truly common, local molecular denominators of pathological changes to vascular endothelium, with a marked distinction of endothelial phenotype between early and advanced plaques.

20 Inactivation of Macrophage Fatty Acid Synthase Decreases Atherosclerosis

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Fatty acid metabolism is disturbed in atherosclerotic lesions but whether it affects lesion formation is unknown. To test the hypothesis that fatty acid synthesis affects atherosclerosis, we inactivated macrophage fatty acid synthase (FAS) in apoE-deficient Cre-Lox mice. FASKOM (FAS KnockOut in Macrophages) animals showed essentially no FAS mRNA or enzyme activity in macrophages. FASKOM mice and their wild type littermates on the apoE-null background were fed Western diet for 20 weeks. There was no effect of genotype on body weight, glucose metabolism or serum lipids in either sex. FASKOM mice compared to controls had significantly lower systolic and diastolic blood pressures both at baseline on chow diet (92±7.3 vs 102±7.8; p<0.05) and with high fat feeding (100±8.7 vs 126±2.9; p<0.05). Compared to littermate controls (n=17), FASKOM mice (n=21) had 40% less atherosclerotic burden in the abdominal aorta (p<0.001), 20% in the aortic arch (p<0.05), and 23% less in the thoracic aorta (p<0.05). In other tissues, FAS is necessary for endemic activation of the nuclear receptor PPARalpha and expression of its target genes. In elicited macrophages from FASKOM mice, there was no effect on PPARalpha mRNA (as expected), but expression of the PPARalpha target gene MIP11 was reduced (p<0.05) in the FASKOM compared to controls. Treatment of FASKOM macrophages with a PPARalpha agonist normalized the expression of PPARalpha target genes as well as LXRalpha. These results suggest that macrophage FAS promotes atherosclerosis in apoE-deficient mice by decreasing LXRA expression through endogenous activation of PPARalpha.

21 A Cluster of Basic Residues Within the Factor V B-domain Determines Presence of the Procofactor State

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Blood coagulation factor V (FV) circulates as an inactive procofactor protein and must be proteolytically processed to express procoagulant activity. Thrombin is the principal activator of FV and cleaves three peptide bonds, thereby removing a large central B-domain in order to generate active FV (FVa). Mechanistic explanations as to how B-domain release facilitates FV activation are not adequately developed. Recent studies from our laboratory using a panel of F-domain truncated FV variants suggest that B-domain sequences within Ap902-Glu1033 contribute to preserving the procofactor state. Interestingly, the Lys963-Lys1008 portion of this sequence is very basic (18 out of 46 residues are Arg or Lys) and is highly conserved, even though most of the B-domain is poorly conserved within the vertebrate lineage. The aim of the current study was to examine whether this basic region is sufficient to maintain the FV procofactor state. Using the previously characterized and constitutively active FV-810 derivative as scaffold (B-domain sequence Prod11-Gly1491 deleted), we expressed and purified three FV variants with portions of the basic region reinserted: FV-1100SR with Glu986-Glu1077 inserted, FV-1015SR with Lys963-Leu1015 inserted, and FV-1033SR with Lys963-Glu1033 inserted. Each of the proteins migrated as a single band on SDS-PAGE and was processed by thrombin to yield the expected heavy and light chains. Using both a one-stage PT-based clotting assay and a thrombin-induced micro-thrombus model, we show that a B-domain that lacks ~75% of the full length B-domain sequence and structure. Based on our data, we speculate that the basic sequence might serve an inhibitory function, possibly by directly concealing binding interactions that govern the function of the active cofactor species.

22 The Utility of Quantitative Calf Muscle Near-Infrared Spectroscopy in the Follow-up of Acute Deep Vein Thrombosis

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BACKGROUND: To investigate patterns of venous insufficiency and changes in calf muscle deoxygenated hemoglobin (Hb) levels after an acute deep vein thrombosis. METHODS: A total
of 78 limbs with an acute deep vein thrombosis (DVT) involving 156 anatomic segments were evaluated with duplex scanning and near-infrared spectroscopy (NIRS) at 1 month, 3 months, 6 months, and 1 year. Venous segments were examined whether they were occluded, partially recanalized, and totally recanalized, and the development of venous reflux was noted. The NIRS was used to measure calf muscle Hb levels. Calf venous filling index (HHbFI) was calculated by digit spanning, then the HHbRI was calculated by the venous ejection index (HHeRI) and the venous retention index (HHbRI) were obtained after exercise. RESULTS: The segments investigated were the common femoral vein (CFV; 38 segments), femoral vein (FV; 37), popliteal vein (POPV; 44), and calf veins (CV; 37). At 1 year, thrombi had fully resolved in 67% of the segments, 27% remained partially recanalized, 6% were occluded. The venous occlusion was predominant in the FV (24%) at 1 year. On the contrary, rapid recanalization was obtained in CV than proximal veins at each examination (p<0.01). Venous reflux was predominant in POPV (55%), followed by FV (19%), and no reflux was found in CV. At 1 year, the HHeRI in POPV reflux patients was significantly higher (p<0.05) than complete resolution (0.011, respectively). Similarly, there was a significant difference in the HHbRI between the two groups (1.42, 1.56, p<0.05, respectively). The lower HHeRI and HHbRI groups show different proportions of occlusion, partial recanalization, and total recanalization. The CV shows more rapid recanalization than proximal veins. The NIRS-derived HHbRI and HHbRI could be promising parameters as the overall venous function in the follow-up of acute DVT. These findings might be very helpful for physician in detecting patients who require much longer follow-up studies.

A Unique Function for Low-density Receptor–Related Protein-1: A Component of a 2-Receptor System Mediating Specific Endocytosis of Plasma-derived Factor V by Megakaryocytes

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Factor V is endocytosed by megakaryocytes from plasma via a specific, receptor-mediated, clathrin-dependent mechanism to form the functionally and physically unique platelet-derived factor V pool. Because of its ability to endocytose proteins involved in hemostasis, the role of low density lipoprotein receptor-related protein-1 (LRP-1) in factor V endocytosis by the megakaryocyte–like cell line, CMK, was examined. Equilibrium binding of 125I-factor V to CMK cells is defined by a sigmoidal binding isotherm, suggesting that factor V binding is cooperative and clathrin mediated and is cooperative and mediated by a two-receptor system. Furthermore, 125I-Factor V binding is reversible and partially sensitive to receptor associated protein (RAP), a known LRP-1 ligand. Based on these observations, a two-receptor model for factor V binding to megakaryocytes was hypothesized. In this model, factor V binds to a specific receptor facilitating binding of another factor V molecule to LRP-1 or a like molecule, which subsequently endocytoses factor V. The purpose of the current study was to identify which member of the LRP-1 receptor family is involved. Using RT-PCR, expression of an LRP-1 transcript in CMK cells was demonstrated. In contrast, transcripts representing other LRP family members could not be identified. Cell surface expression of LRP-1 antiserum by CMK cells was confirmed by flow cytometry using polyclonal anti-LRP-1 antibodies. Greater than 70% of the CMK cells expressed LRP-1 on their cell surface. Co-localization of endocytosed AlexaFluor488-factor V and LRP-1 demonstrated that all of the factor V positive cells expressed LRP-1. These same anti-LRP-1 antibodies were used to displace ~40% of the bound 125I-factor V from the surface of the cells. Furthermore, factor VIII, a known ligand of LRP-1, inhibited 125I-factor V endocytosis equally as well as factor V when present at a 25-fold molar excess. These combined observations confirm our model of the two-receptor system. These events regulating factor V binding to V and RAP represent a novel paradigm whereby an essential coagulation protein is endocytosed from plasma and modified intracellularly to yield a functionally distinct molecule. Furthermore, a role for LRP-1 in endocytosis of a protein not destined for lysosomal degradation is unique.

Induction of Tissue Factor and Loss of Thrombomodulin Activities upon Inflammatory Stimulation in Vivo

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Introduction: Inflammation induces organ specific changes in expression of tissue factor (TF) and thrombomodulin (TM) antigens, but it is unknown whether this translates to a net procoagulant phenotype in vivo. Hypothesis: induction of TF and suppression of TM activities occurs in inflamed organs—aroused by endotoxemia in specific visceral segments including endotoxin (LPS) and particulate matter (PM). Design and results: In C57Bl/6 mice TFactivity in the lungs was induced more by intraperitoneal LPS (25.8 ± 5.3 pm) than by saline (12.7 ± 1.7 pm; p<0.05); TF activities in other organs (brain, heart, spleen, liver, kidney) were comparable for LPS and saline. Addition of lung homogenate from control mice to plasma markedly reduced the observed thrombin potential (ETP) (175 ± 61 vs. 1437 ± 112 nmM; p<0.01). This inhibitory effect was due to TF in the lungs, because it was absent in protein C deficient plasma; II) lungs from TM-/- mice with a 100-fold reduced potential to activate protein C did not inhibit thrombin generation in plasma (ETP: 1688 ± 209 nMmin); III) TF challenge abolished the inhibitory activity of the lungs, indicated by a significant increase in ETP (941 ± 523 vs. 194 ± 159 nMmin); LPS did not affect other organs in their expression of TF, IL-6 and TNF-α was enhanced in p85α PMs. Furthermore, inflammation and coagulation were enhanced in endotoxemic wild type mice lacking p85α in hematopoietic cells. LPS activation of the MAPKs and the expression of TF, IL-6 and TNF-α were reduced in PTEN-/- PMs. Our results indicate that LPS activation of the FMK pathway in macrophages inhibits the MAPK signaling pathways and reduces inflammation and coagulation. Activation of P3K or inhibition of PTEN may represent novel strategies to reduce inflammation and coagulation in endotoxemia and sepsis.

Angiotensin II-induced Hypertrophy and Fibrosis is Prevented by Angiotensin 1–7 Overexpression in the Heart

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Background: In vitro studies demonstrate that Ang-1–7 opposes many actions of Ang II. Whether similar effects occur in vivo remains unclear. Objectives: We tested the hypothesis that Ang-1–7 protects the heart from damage induced by Ang II and assessed some signaling pathways whereby this occurs. Methods and results: Transgenic mice over-producing Ang-1–7 (TG/Ang-1–7) exclusively in the heart (6–fold) were generated. Basal blood pressure and cardiac contractility were similar between groups. We evaluated whether Ang-1–7 acts direct cardioprotective effects, TG/Ang-1–7 and control mice were infused with Ang II (350ng/kg/min, 19 days). Ang II-infused TG/Ang-1–7 mice developed less ventricular hypertrophy and fibrosis versus controls, in spite of developing similar levels of hypertension (p>0.05). Increased myocardial expression of the NAD(P)H oxidase subunit p39, an index of activity, was observed in Ang II-infused controls. This was blunted in TG/Ang-1–7 mice. Phosphorylation of cardiac c-Src was increased by Ang II in controls (2.5-fold) but not in TG/Ang-1–7 mice. Activation of redox-sensitive growth signaling molecules, Akt and p38MAPK, was increased by Ang II in controls (2.5-fold, p<0.001) without effect in TG/Ang-1–7 mice. Ang II increased cardiac ERK 1/2 phosphorylation in control and TG/Ang-1–7 mice. Effects of Ang-1–7 overproduction on cardiac cell cycle regulatory proteins were also assessed. Expression of Cdk2, Cyclin E and Cyclin D/Cdk4 was increased in Ang II-infused controls (1–2-fold) and abrogated in TG/Ang-1–7. Increased expression of cell cycle inhibitors p27 (1–4-fold) and p53 (1.5-fold) by Ang II was observed in controls but not in TG/Ang-1–7 mice. p27 expression was not different between groups. Conclusion: Our data indicate that Ang-1–7 protects the heart from an Ang II-dependent hypertensive challenge. This is associated with downregulation of NAD(P)H oxidase, c-Src, Akt and p38MAPK, but not with ERK1/2. Moreover, Ang-1–7 influences cell cycle regulatory proteins. Our findings suggest that the negative cardiomyocyte signaling pathways whereby potentially deleterious actions of Ang-1–7 may represent a protective mechanism whereby potentially deleterious actions of Ang-1–7 are counterbalanced.

Requirement of RhoA Serine 188 Phosphorylation for cGMP Kinase-mediated Vascular Protection

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[Background] cGMP agonists such as natriuretic peptides dilate blood vessels and inhibit vascular remodeling. Small GTPase RhoA and its effector ROCK, crucial mediators of
Matrix Metalloprotease-1: Role in Aneurysm Formation in Vivo and Regulation by Nicotine in Vascular Smooth Muscle Cells

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The role of matrix metalloprotease-1 (MMP-1) and the molecular mechanisms regulating its expression in aneurysm remain unknown. Transgenic mice expressing human MMP-1 in macrophages were crossed into the ApoE-null / Timp-1 (Tissue inhibitor of metalloprotease-1) knockout background, which develops a high number of microanatomy associated with atherosclerotic plaque formation. Transgenic and non-transgenic controls (n=10) were given a high-fat diet for ten weeks and sacrificed. Transgenic mice had larger aneurysms (45,216±28,111 μm3, n=33) compared to the controls (26,185±22,026 μm3, n=28, P<0.005). These aneurysms were also characterized by the bulging of the dissection plane, leading into the adventitia. Aortic media histology revealed: 32±14% of the aneurysm area, controls: 12±5%, n=11 in each group; P<0.005, a marker of severity in the human disease. Since smoking is a major risk factor for aneurysm development and rupture, we tested the hypothesis that nicotine could modulate the expression of MMP-1 in vascular cells. Human aortic smooth muscle cells in treated with nicotine, at concentrations found in the circulation of moderate smokers (10 nm to 1000 nm). After 24 hours, nicotine increased the expression of MMP-1, both at the mRNA and protein levels (P<0.05). This up-regulation was abrogated by specific inhibitors of p38 and ERK, suggesting that nicotine up-regulates MMP-1 through the MAP kinases cascade transduction pathway. Western-blot analysis of cell lysates showed that nicotine activates the Jak/STAT kinase pathway, leading to increased phosphorylation of Jak2, ERK, p38, Jnk, and Stat3, and subsequent induction of MMP-1 expression. Our data demonstrates that MMP-1 enhances the severity of mouse aneurysm formation, and that nicotine induces MMP-1 expression in smooth muscle cells through the Jak/Stat kinase pathway. This study suggests that increased susceptibility to aneurysm formation and rupture in smokers might result from augmented collagenase activity in the vessel wall, due to a chronic exposure to circulating nicotine.

Notch Signaling Negatively Regulates Endothelial Lineage Specification

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Endothelial cells and hematopoietic cells are thought to arise from a common progenitor, the hemangioblast. This notion was first suggested as a result of the observations that two cell types develop in close proximity during embryonic development. Although the data acquired from cell culture experiments suggest the existence of the hemangioblast, the presence of bipotential progenitors has yet to be demonstrated in vivo until our recent study. We have reported the first in vivo evidence for the existence of hemangioblast during embryonic development using zebrafish gastrula as a model system. As the initial step to delineate the molecular signaling pathways that regulate hemangioblast lineage specification, we analyzed the function of Notch signaling pathway in this process. Previous study indicated that Notch could function as a b-modal switch in the hemangioblast equivalent cell population in Drosophila. We find that Notch signaling appears to promote hematopoietic fate over endothelial fate. Embryos with reduced Notch activity show a significant increased number of angioblasts at the expense of hematopoietic progenitors. This early requirement of Notch activity can be separated from the previously described function of Notch in promoting arterial endothelial fate. Furthermore, by using lineage tracing we show that a certain population of cells which would give rise to primitive erythrocytes translocate into endothelial precursors in embryos with compromised Notch signaling, Although further analyses need to be done to test whether the increased number of angioblasts is due to the fate changes within the hemangioblast lineage or the direct transformation of hematopoietic progenitors, our data suggest that Notch signaling might functions as a cell fate switch by negatively regulating endothelial fate.

The Atheroprotective Effect of Flk-1-based DNA Vaccination in LDL Receptor–Deficient Mice Is Enhanced by Coexpression of CCL21

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Suppression of angiogenesis can reduce plaque neovascularization and inhibit atherosclerotic lesion progression. Oral vaccination of hyperlipidemic LDL receptor-deficient (LDLr-/-) mice with a murine DNA vaccine specific for the murine VEGF receptor 2 (Flk-1) evoked a CD8+ T cell-mediated response that suppressed atherosclerosis-related angiogenesis and attenuated lesion progression. Appropriate adjuvants can improve effector T cell function and increase the efficacy of DNA vaccines. We therefore tested the hypothesis that coexpression of the secretory chemokine CCL21 with Flk-1 can enhance the therapeutic effect of Flk-1 DNA vaccination. Empty expression vector, Flk-1 or Flk-1/CCL21 vaccinated LDLr-/- mice (n = 8/group) were fed a high fat diet for 16 weeks. Consistent with previous findings, there was no difference in total cholesterol levels between all groups of mice, despite the expected increase in plasma cholesterol after high fat diet consumption. Compared to both Flk-1 and control immunized animals, immunization with the vector encoding Flk-1/CCL21 led to increased in vitro lysis of murine endothelial target cells expressing Flk-1 by T cell-enriched splenocytes. Co-expression of CCL21 led to increased protection against diet-induced atherosclerosis in both male and female mice. Using aortic root and tail artery cross sections, we demonstrated reduced neovascularization and macrophage infiltration in aortic segments within areas of disturbed blood flow were altered by the Flk-1-based DNA vaccination at early stages of the disease.

Cyclooxygenase-2 Expression Increases Vascular Inflammation and Abdominal Aortic Aneurysm Formation in Mice

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Abdominal aortic aneurysms (AAAs) are associated with a profound inflammatory response within the vessel wall throughout propagation of the disease. Increased expression of cyclooxygenase-2 (COX-2) is suggested to contribute to the disease in humans. We examined the hypothesis that COX-2-dependent proliferation or inflammation in the abdominal aorta contributes to AAA formation. AAAs were induced by angiotensin II infusion and compared between COX-2-deficient and mice with wild-type littermate controls. COX-2-deficient mice showed significantly reduced AAA incidence at multiple time-points following angiotensin II infusion (day 3, 31% ± 5% for COX-2-/- versus 6% ± 2% for COX-2+/, P < 0.05; day 7, 43% ± 6% for COX-2-/- versus 12% ± 3% for COX-2+/, P < 0.01; day 21, 37% ± 4% for COX-2-/- versus 4% ± 2% for COX-2+/, P < 0.01; day 28, 34% ± 5% for COX-2-/- versus 0% ± 0% for COX-2+/, P < 0.01). COX-2 has previously been shown to be required for angiotensin II-induced smooth muscle cell proliferation. To examine the role of proliferation in COX-2-dependent AAA formation, we determined the effect of COX-2 expression on activation of Akt and Erk1/2. As determined by western blot, the levels of phosphorylated Akt or Erk1/2 were not significantly different between angiotensin II-infused COX-2-/- and COX-2+/- mice, suggesting that COX-2 does not contribute to altered proliferation during AAA formation. Angiotensin II-induced AAA formation is initially characterized by macrophage infiltration into the wall of the abdominal aorta. Therefore, we examined macrophage infiltration as a potential mechanism by which COX-2 contributes to AAA formation. At time-points where angiotensin II was shown to induce COX-2, mRNA expression of the macrophage marker CD68 was significantly attenuated in the COX-2+/- mice, as compared to wild-type controls (for COX-2-/-, versus 6% ± 2% for COX-2+/-, P = 0.07; day 7, 43% ± 6% for COX-2-/- versus 12% ± 3% for COX-2+/, P < 0.01; day 21, 37% ± 4% for COX-2-/- versus 4% ± 2% for COX-2+/, P < 0.01; day 28, 34% ± 5% for COX-2-/- versus 0% ± 0% for COX-2+/, P < 0.01). Furthermore, abdominal aortas of COX-2-/- mice showed attenuated expression of the chemokines MCP-1 and MIP-1alpha, in conclusion, increased COX-2 expression in the abdominal aorta may contribute to AAA formation by enhancing macrophage recruitment.

Integrin Signaling is Critical for Pathological Angiogenesis

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The process of postnatal angiogenesis plays a crucial role in pathogenesis of numerous diseases, including but not limited to tumor growth/metastasis, diabetic retinopathy, and in tissue remodeling upon injury. However, the molecular events underlying this complex process are not well understood and numerous issues remain controversial, including the regulatory function of integrin receptors. To analyze the role of integrin phosphorylation and signaling in angiogenesis, we generated knock-in mice that express a mutant beta3 integrin unable to undergo tyrosine phosphorylation. Two distinct models of pathological angiogenesis revealed that vascular leakage is impaired in mutant beta3 knock-in mice. In an ex vivo angiogenesis assay, mutant beta3 knock-in endothelial cells did not form complete capillaries in response to vascular endothelial growth factor (VEGF) stimulation. At the cellular level, defective tyrosine phosphorylation in mutant beta3 knock-in cells resulted in impaired adhesion, spreading, and migration of endothelial cells. At the molecular level, VEGF-stimulated transformation between VEGF receptor-2 and beta3 integrin in wild-type but not in mutant beta3 knock-in endothelial cells. Moreover, phosphorylation of VEGF receptor-2 was significantly reduced in cells expressing mutant beta3 compared to wild type, leading to impaired integrin activation in these cells. These findings provide novel mechanistic insights into the role of integrin-VEGF axis in pathological angiogenesis.
conclusion, these studies demonstrated that co-expression of CCL21 with Fk-1 improved T cell mediated immune responses against Fk-1 and reduced lesion progression in hyperlipidemic mice.

Diet-induced Dyslipidemia Is Associated with Acceleration of Disease and Mortality in Lupus-susceptible LDLr<sup>−/−</sup> Mice

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Individuals suffering from systemic lupus erythematosus (SLE) are predisposed to accelerated atherosclerosis. Unfortunately, the underlying mechanisms for increased vascular disease in lupus are not well understood. Our laboratory has recently developed an animal model of SLE-accelerated atherosclerosis. We have shown that radiation chimeras consisting of SLE-derived hematopoietic cells transferred to LDLr<sup>−/−</sup> mice have increased atherosclerosis. Surprisingly, feeding mice high-fat diet for 8 weeks resulted in significant mortality in SLE-susceptible mice compared to controls. Based on these data, we hypothesized that increased dyslipidemia was characterized by an accumulation of non-HDL lipoproteins, which is associated with increased atherosclerosis. To test this hypothesis, we created radiation chimeras of LDLr<sup>−/−</sup> mice that were either SLE-susceptible (DLR.Sle) or resistant (DLR.B6). Eight weeks following bone marrow reconstitution, mice were placed on a normal chow or high fat (21% fat, 0.15% cholesterol) diet for eight weeks. All animals fed a high-fat diet had significantly increased total cholesterol and triglycerides compared to chow fed mice, however there were no significant differences within groups between DLR.B6 control and DLR.Sle mice. Compared to all chow-fed animals and high-fat fed DLR.B6 controls, high-fat fed DLR.Sle mice exhibited increased mortality (37%) and were mildly, but significantly hypertensive. In addition, ECHO analyses showed that 60% (3 of 5 mice) of the DLR.Sle mice fed high fat diet had increased left ventricular mass compared their DLR.B6 counterparts. Increased blood pressure did not overtly appear to be due to advanced renal disease as serum creatinine and urea levels between DLR.Sle mice on chow or high-fat diet did not differ significantly. These data demonstrate that increased dyslipidemia resulting from feeding a high-fat diet can exacerbate atherosclerosis and associated vascular complications.

Chemical Genetic Analysis Reveals the Central Role of Phosphatidylinositol-3 Kinase and MAP Kinase/ERK Signaling Pathways in Artery/Vein Specification

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How the endothelial progenitor cells are specified to either the arterial or venous fates is a fundamental biological question with significant clinical implications. We reasoned that the arterial specification during development is amenable to chemical genetic analysis, in a manner analogous to the classical genetic analyses, which have been instrumental in elucidation of numerous biological pathways in prokarocytes and intervebriles. Specifically, we hypothesized that small molecules found to suppress zebrafish model of arterial deficiency will function by augmenting the signaling for arterial specification. In a high-throughput chemical screen, we discovered two classes of small molecules that rescued the defective arteriogenesis caused by a genetic mutation. Using these compounds as chemical tools to dissect the signaling pathways involved in artery/vein specification, we made the following important and surprising observations: 1) p42/p44 MAP kinase (ERK) activation is a specific and early marker with arterial progenitors, 2) ERK activation is a key determinant of arterial specification, and 3) the phosphatidylinositol-3 kinase (PI3K) has the opposite effect on artery-vein specification. In summary, our chemical genetic approach has revealed opposing roles of PI3K and ERK in artery/vein specification, a better understanding of which will lead to novel therapies for numerous conditions, such as ischemic heart/limb diseases and vascular malformations.

ER Stimulators Promote TLR4/TLR4-dependent Macrophage Apoptosis Through Ca<sup>2+</sup>-mediated CaMKII Activation

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The macrophage (Mo) scavenger receptor-A (SRA) and toll-like receptor 4 (TLR4) are pattern recognition receptors (PRRs) of the innate immune system. In two-data suggest that the SRA and TLR4 contribute to the development of atherosclerosis, although the mechanistic links between these PRRs and atherosclerosis have not been elucidated. Our laboratory has focused on a critical event in the formation of necrotic atherosclerotic plaques, namely, Mo-death. We recently discovered that SRA ligands trigger Mo-apoptosis in an SRA- and TLR4-dependent manner. SRA ligands activate the pro-apoptotic TLR4-JNK pathway but, unlike PI3K, silence the pro-survival TLR4-FGlu pathway. We also found that ER stressors that induce Ca<sup>2+</sup>-mobilization enhance TLR4-dependent signaling and that chelating Ca<sup>2+</sup> inhibits TLR4-dependent JNK activation and apoptosis. In the current study we show that LPS, which normally fails to cell surface receptors, induces Mo apoptosis in response to various SRA ligands such as fucoidan, β-amyloid, and advanced glycation endproducts (AGEs). These SRA ligands do not induce apoptosis when added in the absence of LPS. Moreover, Mo-apoptosis induced by LPS and SRA ligands was markedly amplified in the setting of Ca<sup>2+</sup>-releasing ER stressors such as thapsigargin. To probe mechanism, we investigated the role of Ca<sup>2+</sup>-regulated calcium channels and a dependent protein kinase II (CaMKII). We found that apoptosis-enhancing Ca<sup>2+</sup>-releasing ER stressors contribute to two events necessary for Mo-death: enhancement of TLR4-dependent JNK activation through activation of CaMKII and induction of the pro-apoptotic CHOP branch of the ER stress pathway through depletion of ER Ca<sup>2+</sup> stores. These findings reveal a novel link between ER stress, Ca<sup>2+</sup> mobilization, and PRR signaling and have potential implications for Mo-death and plaque necrosis in advanced atherosclerosis and perhaps other diseases where PRRs, ER stress, and cell death could play a role.

Signal-Dependent Splicing of Tissue Factor Pre-mRNA Modulates the Thrombogenicity of Human Platelets: A New Mechanism Linked to Disordered Coagulation in Sepsis

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Introduction: We recently demonstrated that platelets use a previously-unknown pre-mRNA splicing pathway to generate tissue factor (TF)-dependent procoagulant activity. Here, we hypothesize that TF pre-mRNA splicing events are activated in patients with sepsis, a syndrome of dysregulated coagulation. Methods: Patients meeting consensus criteria for sepsis were prospectively enrolled. Platelets were freshly-isolated from whole blood within the first 24 hours of admission to the ICU. Platelets from non-sepsis and healthy controls were assayed in parallel to eliminate differences due to inter-assay variability. TF mRNA expression patterns and procoagulant activity was measured in both groups. Platelets were also incubated with bacteria from septic patients or bacterial-derived products. Results: Without exception, platelets from healthy controls (n=54) expressed TF pre-mRNA. In contrast, platelets from 26 of 32 (81%) septic patients expressed TF pre-mRNA. The percentage of TF pre-mRNA splice products increased with increasing severity of illness as measured by APACHE II scores. Consistent with TF mRNA expression patterns, procoagulant activity in platelets from septic patients was significantly (p<0.05) higher than in healthy controls (46.5 ± 13.3 vs. 11.1 ± 2.8 pM). In a subgroup of patients (n=16), we also assessed TF pre-mRNA splicing in serial samples. We found progressive splicing in the platelets over time. Altogether, 81% of these patients expressed spliced TF mRNA at some point during their ICU stay. Gram (E. coli) or gram+ (S. aureus) bacteria isolated from septic patients also induced TF pre-mRNA splicing. Similarly, products of E. coli (lipopolysaccharide) or S. aureus (α-toxin) induced TF pre-mRNA splicing in platelets, resulting in accelerated clot formation. Conclusions: These data demonstrate that circulating platelets from septic patients spontaneously splice TF pre-mRNA and generate procoagulant activity. TF pre-mRNA splicing by platelets may contribute to abnormal coagulation in sepsis and may be a target of future therapeutics.

Targeting Coagulation Factor XII Provides Protection from Pathological Thrombus Formation in Myocardial Infarction and Cerebral Ischemia Without Interfering with Hemostasis

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Formation of fibrin is critical for limiting blood loss at a site of blood vessel injury (hemostasis), but may also contribute to vascular thrombosis. Hereditary deficiency of factor XII (FXII), the protease that triggers the intrinsic pathway of coagulation in vitro, is not associated with spontaneous or excessive injury-related bleeding, indicating FXII is not required for hemostasis. We demonstrate that deficiency or inhibition of FXII protects mice from ischemic brain injury and cardiac ischemia/reperfusion damage. Following transient middle cerebral artery occlusion, the volume of infarcted brain in FXII deficient and FXII inhibitor-treated mice was significantly less than in wild type controls, without an increase in infarct-associated hemorrhage. FXII-null and inhibitor treated mice were largely protected from ischemia/ reperfusion injury and survival rate was significantly higher in myocardial infarction models. Targeting FXII reduced fibrin formation in ischemic vessels, and reconstitution of FXII deficient mice with human FXII restored fibrin deposition. Mice deficient in the FXII substrate factor XI were similarly protected from vessel-occluding fibrin formation in brain and heart, suggesting that FXII contributes to pathologic clotting through the intrinsic pathway. These data demonstrate that some processes involved in pathologic thrombus formation are distinct from those required for normal hemostasis. As FXII appears to be instrumental in pathologic fibrin formation, but dispensable for hemostasis, FXII inhibition may offer a selective and safe strategy for preventing stroke, myocardial infarction and other thromboembolic diseases.

The Antithrombotic Potential of Selective Blockade of Tali-dependent Integrin αIβ3 (Platelet GPⅡb-Ⅲa) Activation

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Studies in vitro and with cultured cells indicate that talin binding to the β3 cytoplasmic domain is a final step in platelet integrin αIβ3 (GPⅡb-Ⅲa) activation. We tested the significance of talin-mediated integrin activation by generating platelet-specific talin-1 knockout mice (PKO). Platelets from PKO mice showed a dramatic reduction in agonist-induced αIβ3 activation as determined by soluble fibrinogen binding. In addition, more than 90% of PKO mice showed pathological bleeding that was associated with reduced survival. To determine whether the phenotype of PKO mice was due to lack of talin binding to integrin β3, we generated β3 integrin negative mice having genetically-null αIβ3 integrin cytoplasmic domain that disrupt talin-β3 integrin interactions. We introduced a β3<sup>Y747A</sup> substitution that disrupts the
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Expression and Characterization of Mutants of Human TAFI Resistant to Activation by Specific Proteases

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Thrombin-activatable fibrinolytic inhibitor (TAFI) is a human plasma zymogen that functions as a molecular connection between the coagulation and fibrinolytic cascades. Activated TAFI (TAFIa) is formed by cleavage at Arg92, a reaction that can be catalyzed by several proteases including thrombin and plasmin; thrombin in complex with thrombomodulin (TM) is the most potent activator of TAFI. However, the relative roles of each of these activators in different physiological contexts remains unknown. We have designed mutants of TAFI with the aim of creating variants that are resistant to activation by either thrombin or plasmin. Substitution of serine for proline at position 91 (P91S) yielded a variant of TAFI that could not be activated by thrombin and was only very slowly activated by thrombin-TM; this variant was activated normally by plasmin. The P91S variant was expressed in mammalian cells, purified, and its ability to inhibit lysis of clots made from TAFI-deficient plasma was compared to that of wild-type (wt) recombinant TAFI. TAFI (P91S) was markedly impaired in its antifibrinolytic activity, both in the presence or absence of TM (see Figure). Thus, activation by thrombin and thrombin-TM predominates under these circumstances. Plasmin is a promiscuous enzyme, but is unable to cleave its own activation site; accordingly, introduction of residues from this site into peptide substrates for plasmin prevents their cleavage. However, introduction these residues into analogous positions in TAFI (specifically, valine at positions 93 and 94) failed to affect plasmin activation of TAFI suggesting that exosite interactions are more critical for recognition of TAFI by plasmin.

The Molecular Basis for Fibrin Clot Elasticity

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Fibrinogen when activated by thrombin forms a thrombus having elasticity that buffers blood’s shear forces. The molecular basis of this elasticity is unknown. Fibrinogen has coiled coils which are structural motifs that have been demonstrated in myosin to have perfect elasticity by atomic force microscopy (AFM). ‘Signature’ phases of the coiled coil’s force extension curves have been defined that can distinguish it from other protein domains. Furthermore, ‘polymers’ of fibrinogen molecules could be created for force spectroscopy experiments to amplify the signal from pulling single fibrinogen molecules. Fibrinogen is thus ideal for exploring the force-extension behavior of the coiled coil region for fibrin elasticity. To generate linear polymeric fibrin strands, 70 µl of purified fibrinogen (0.1 mg/ml) was reacted with 10 µl of CaCl2 (2.5 mM), 10 µl of FXII (0.002 mg/ml) and 10 µl of thrombin (0.2 U/ml) for 3.5 minutes. These were then imaged with the AFM in liquid tapping mode. Linear strands of 7 to 10 fibrin monomers with lengths of 250 ± 75 nm were observed. Fibrinogen and fibrin polymers were deposited onto gold-sputtered coverslips and adsorbed for 5 minutes. AFM in force spectroscopy mode was used to stretch the fibrinogen molecules and fibrin polymers. When the fibrinogen molecule was stretched beyond 20 nm, there was an abrupt increase in force of about 60 pN, followed by a plateau phase during which the force was relatively constant. This intermediate conformational transition likely contributes to fibrin elasticity. The magnitude of the plateau force changed with pH and calcium ion concentration. Stretching fibrin polymers to 400 nm revealed a blunted sawtooth pattern of consecutive force peaks, each decreasing the modulus of the coiled coil segments. Increasing the stretch to 3 times their length unfolded the globular domains. Repeated extension and relaxation cycles demonstrated that the coiled coil regions were able to refold at high rates. Our data provide the first evidence for the coiled coils of fibrinogen being the origins of fibrin elasticity. Alterations to the coiled coils through mutations or disease may interfere with its function, changing the elasticity of the fibrin clot and cause thromboembolism.
**Role of LIM Kinase 1 in Endothelial Function**

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Microtubule (MT) destabilization promotes the formation of actin stress fibers and enhances the contractility of cells; however, the mechanism involved in the coordinated regulation of MTs and the actin cytoskeleton is poorly understood. LIM kinase 1 (LIMK1) regulates actin polymerization by phosphorylating the actin depolymerization factor, cofilin. We have shown that LIMK1 is involved in MT destabilization. Over-expression of wild type LIMK1 or MT destabilization, whereas the kinase-dead mutant of LIMK1 (KO) did not affect MT stability. Importantly, down-regulation of endogenous LIMK1 by small interference RNA resulted in abrogation of the thrombin-induced MTs destabilization and the inhibition of thrombin-inactivated actin polymerization. Expression of Rho kinase 2, which phosphorylates and activates LIMK1, dramatically decreases the interaction of LIMK1 with tubulin but increases its interaction with actin. Interestingly, expression of KD-LIMK1 or small interference RNA-LIMK1 prevents thrombin-induced microtubule destabilization and F-actin formation, suggesting that LIMK1 activity is required for thrombin-induced modulation of microtubule destabilization and actin polymerization. We have shown that the stable expression of the LIMK1 knockout mice, we found that endothelial permeability in the lungs of LIMK1 +/- mice was lower than that of wild type mice. Perfusion of the lungs of wild type mice with PAR1 peptide showed significant increase of endothelial permeability. Notably, the endothelial permeability of the lungs of LIMK1 +/- mice after PAR1 peptide stimulation was significantly lower than that of wild type. Acute lung injury (ALI) is a syndrome of acute respiratory failure that results from acute pulmonary edema and inflammation. Using lipopolysaccharide (LPS) injection as a model of ALI, we have shown that LIMK1 +/- mice did not develop lung edema and showed significantly reduced mortality as compared with wild type mice. Our findings indicate that LIMK1 coordinates microtubules and actin cytoskeleton. We suggest that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels.

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**Distinct eNOS Regulation by Protease-activated Receptors Involving G12/13 and Rho-kinase**

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Protease-activated receptors (PARs), such as PAR-1 and PAR-2 have been implicated in the regulation of endothelial nitric oxide (NO) production. We hypothesized that PAR-1 and PAR-2 distinctly regulate the activity of endothelial NO synthase (eNOS) through the selective phosphorylation of a positive regulatory site, Ser1179 and a negative regulatory site, Thr497. In bovine aortic endothelial cells (BAECs), a PAR-1 ligand, TFLRR, phosphorylated eNOS at Thr497. It had no effect on Ser1179 phosphorylation or cyclic GMP (cGMP) production. In contrast, a PAR-2 ligand, SLIGRL, phosphorylated Ser1179 with no noticeable effect on Thr497. SLIGRL stimulated cGMP production that was blocked by L-NAME. Neither eNOS phosphorylation nor cGMP production was observed by a PAR-4 agonist, AT2-NH2. Thrombin has been shown to transactivate PAR-2 through PAR-1. Thus, thrombin stimulates eNOS phosphorylation at both sites as well as cGMP production in BAECs. Importantly, SLIGRL-induced Ser1179 phosphorylation and cGMP production were inhibited by a Gg antagonist, YM-254890. YM-254890 also blocked thrombin-induced cGMP production and eNOS phosphorylation at Ser1179 and Thr497, demonstrating that eNOS phosphorylation and cGMP production were mediated by PAR-2 and not PAR-1. Furthermore, eNOS phosphorylation was not affected by Gi inhibitors (over-expression of GRK2 C-tail and pertussis toxin pretreatment). By contrast, TFLLR-induced phosphorylation and cGMP production were inhibited by a Gq inhibitor, YM-254890. YM-254890 inhibited tubule formation of HUVECs on matrigel vs. adenoviral infected controls (n=6, p<0.01). Similarly, there was a significant decrease in cellular proliferation in Sprouty1 overexpressing HUVECs (p<0.01). A promoter analysis revealed three putative HF-1a binding sites in the Sprouty1 gene. Using human umbilical vein endothelial cells (HUVECs), Sprouty1 protein expression was significantly increased after exposure to 0.2% oxygen for 24 hours. Sprouty1 overexpression significantly inhibited the formation of HUVECs on matrigel vs. adenoviral infected controls (n=6, p<0.01). Similarly, there was a significant decrease in cellular proliferation in Sprouty1 infected HUVECs, as determined by BrdU incorporation (n=6, p<0.01). Interestingly, Sprouty1 is sufficient to increase p21 protein expression, a well-known cell cycle inhibitor. HUVECs under hypoxia with O2 concentrations (0.2% vs. 21% O2) showed decreased p21 protein expression at 24 hours. In summary, we have identified a novel conserved tyrosine kinase signaling pathway that inhibits angiogenesis and may have important therapeutic implications in cardiovascular disease.

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**β-Arrestin-2 is Required for B1 Kinin Receptor-dependent Activation of iNOS**

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B1 kinin receptor stimulation leads to the recruitment and scaffolding of β-arrestin-2, facilitating iNOS phosphorylation, activation and production of "super-high output" NO. This B1R signaling pathway may be important in regulating endothelial barrier function during inflammation and may also play a role in the beneficial therapeutic effects of ACE inhibitors, which can also act as direct agonists of B1Rs.

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**Rap1b-deficiency Leads to Decreased Angiogenesis, Endothelial Cell Proliferation, Migration, and MAPK Signaling**

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Introduction Small GTPase Rap1 regulates basic cellular functions: cell-matrix and cell-cell adhesion, migration, differentiation and growth. We described murine Rap1b-knockout (KO) adult phenotype, which includes a mild platelet agregation defect and protection from thrombosis in vivo. More striking is the embryonic phenotype, as the majority of Rap1b-KO mice suffer from embryonic bleeding resulting in embryonic and perinatal mortality. Hypothesis The severity of the embryonic defects is due to a smaller size of surviving KO mice, therefore less KO embryos are not required for embryo hemostasis indicated a defect in vascular development. However, gross vascular patterning of KO embryos was normal. Thus, we assessed the hypothesis that Rap1b-KO mice suffer a defect in angiogenesis, the main mechanism of vascular remodeling during late development and after birth. Methods Angiogenesis in vivo was analyzed in a neonatal retinal model. To determine if the defect is inherent in blood vessels, aortic ring sprouting assay was performed. Angiogenic responses of endothelial cells (ECs) were assessed; proliferation in vivo and migration and biochemical activation of primary lung ECs in response to VEGF and PKH26 in HUVECs and in vitro. Results and Discussion We showed that B1R-dependent NO production is dependent on β-arrestin-2 recruitment and scaffolding. Indeed, over-expression of β-arrestin-2 significantly increased NO production in response to the B1R agonist, acetylcholine. We observed that B1R-dependent NO production is dependent on β-arrestin-2 recruitment and scaffolding. Indeed, over-expression of β-arrestin-2 significantly increased NO production in response to the B1R agonist, acetylcholine.
Monocyte chemoattractant protein-1 directs migration of monocytes from the peripheral blood to vascular inflammation and is known to play a role in the development of atherosclerotic lesions. The distribution of the monocyte subtypes that mediate this migration have not been rigorously addressed and few studies address whether in vivo observations obtained under controlled conditions can indeed be substantiated in dynamic in vivo situations. Earlier we reported that iPLA2 and cPLA2 are both required for monocyte chemotaxis to MCP-1 and act in parallel. We also proposed that due to the identity of different end products and inability to influence each other’s activity, these enzymes might regulate distinct characteristics of migrating monocytes, probably, from different intracellular locations. In this study, we report that MCP-1 induces recruitment of these two phospholipases to different intracellular locations: iPLA2 to the endoplasmic reticulum and cPLA2 to the Golgi. This differential spatial distribution is manifested also in their functional independence. Monocytes deficient in cPLA2 displayed reduced speed, whereas in contrast, iPLA2 deficient monocytes make wider and more frequent turns as well as exhibiting reduced speed. Thus, iPLA2 provides a directional cue or compass supporting migration toward the MCP-1. We validated the contribution of these phospholipases in migration of monocytes in vivo using a newly developed mouse model. Adaptively transferred murine monocytes, if rendered deficient in either of these phospholipases by their specific transgenic inactivation, or a MCP-1-dependent process. We have identified a previously unknown function of iPLA2 as a cellular compass and present a new approach for evaluating the relevant contributions of signaling molecules in regulating monocyte chemotaxis to MCP-1, in vivo.

Macrophage Phenotype in Atherosclerotic Human Coronary Arteries is Different from Macrophage Phenotype in Normal Human Coronary Arteries

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We previously characterized the divergent atherogenic potential of human macrophage-derived macrophages differentiated with either M-CSF (Mac-M) or GM-CSF (GM-Mac) in vitro. Gene expression analysis and cytokine secretion assays indicated significant differences between the two macrophage phenotypes. The in vitro disease process induced in macrophages by GM-Mac appeared to be more inflammatory while GM-Mac show increased expression of genes related to cholesterol efflux. Immunocytochemistry showed that both macrophage phenotypes express the pan-macrophage marker CD68 while only Mac-M express the pro-inflammatory marker CD14. In the present study, we determine the macrophage phenotype in normal and atherosclerotic human coronary arteries. In accordance with our in vitro findings, CD68+ /CD14+ cells were similar to the Mac-M phenotype and CD68+ /CD14+ cells were similar to the GM-Mac phenotype. Both Mac-M and Mac-M phenotypes were present in normal intimal regions and atheromatous lesions of coronary arteries. Immunostaining of CD68+ and CD14+ cells within the aortas of controls and uninvolved intima from atherosclerotic lesions in vivo demonstrated that Mac-M were more prevalent in atherosclerotic lesions (75 ± 4%) compared with normal intimal regions of coronary arteries (39 ± 6%). In contrast, Mac-M were more prevalent in normal intimal regions (54 ± 1%) compared with atherosclerotic lesions of coronary arteries (21 ± 5%). The immunostaining also revealed the presence of CD68+ /CD14+ cells throughout the adventitia in both normal and diseased regions of the coronary arteries. Cells with CD68+/CD14+ immunolabeling were not present within the media of normal coronary artery. However, media of human coronary arteries with advanced atherosclerotic lesions showed increased migration of CD68+/CD14+ cells from the adventitia through the media to the intima. The predominance of Mac-M in atherosclerotic lesions suggests that this macrophage subpopulation promotes development of these lesions. The identification of macrophage phenotypes with different gene expression patterns and different potentials for promoting atherosclerosis has important experimental and clinical implications.

CXC6 Promotes Atherosclerosis by Supporting T-cell Homing, Interferon γ Production, and Macrophage Accumulation in the Aortic Wall

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T cells are important in atherosclerosis, but very little is known about the mechanisms behind lymphocyte recruitment into atherosclerosis-prone aortas. We tested the hypothesis that CXC6, a chemokine that is expressed on a subset of T cells and natural killer T cells, is involved in lymphocyte homing into the aortas and modulates atherosclerosis. To investigate the role of CXC6 in the atherosclerosis, we bred CXC6-deficient (CXC6KO/ko) mice with apolipoprotein E-deficient (Apoe−/−) mice. CXC6KO/ko/ Apoe−/− mice fed a western diet for 17 weeks or a chow diet for 56 weeks had decreased atherosclerosis compared with Apoe−/− controls. Flow cytometry analysis of the aortas from CXC6KO/ko/ Apoe−/− mice showed that the reduction of atherosclerosis was accompanied by a decreased percentage of CXC6KO/ko/ Apoe−/− T cells within the aortas. Short-term homing experiments demonstrating that CXC6 is involved in the recruitment of CXC6KO/ko leukocytes into the atherosclerosis-prone aortic wall. The reduced percentage of CXC6KO/ko T cells within the aortas was associated with diminished production of IFNγ and reduced accumulation of CD11b+/CD68+ macrophages in the aortas. To address the potential contribution of our observation to the understanding of the role of CXC6 in atherosclerosis, we performed experiments using CXC6KO mice in human carotid endarterectomy specimens and found CXC6KO expressing T cells and some MoMa-2+ CXC6KO+ within the analyzed carotid tissues. These data provide evidence for a functional role of CXC6 in atherosclerosis by altering the recruitment of CXC6KO leukocytes and modulating of the local immune response within the aorta.

Serum Amyloid A Is an Endogenous Ligand for Toll-like Receptor 2

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Serum amyloid A (SAA) is an apolipoprotein produced by hepatocytes during acute-phase response and by other inflammatory cells such as macrophages. SAA is an important clinical indicator of atherosclerosis and other inflammatory diseases, and contributes to cholesterol metabolism through its binding to high-density lipoprotein (HDL) and scavenger receptors. In phagocytes, SAA is a potent inducer of proinflammatory cytokines such as MCP-1, IL-1β, IL-8, and IL-12p40. The observation that SAA could induce cytokine production independently of formyl peptide receptor-like 1 and the scavenger receptor SR-BI led us to propose the presence of another receptor. To assess this hypothesis, we analyzed the cytokine production profiles of SAA stimulated monocytes cells as a tool to understand inflammatory processes. But the role of SAA in the development of atherosclerosis is only little known.

Glutamate Mediates Platelet Activation Through the AMPA Receptor

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Glutamate is an excitatory neurotransmitter in the central nervous system (CNS) that binds to the kainate receptor, the N-methyl-D-aspartate (NMDA) receptor, and the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor (AMPAR). Each receptor was first characterized and cloned in the CNS. Glutamate is also present in the periphery, and glutamate receptors have been identified in non-neuronal tissues, including bone, heart, kidney, pancreas, and platelets. Platelets have a central role in normal thrombosis and hemostasis as well as connective tissue disease process.

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Hematopoietic Lineage Cell-specific Protein-1 (HS1) is an Important Signaling Molecule Downstream from Protease-activated Receptors and Is Involved in Multiple Signaling Pathways of Platelet Activation

Bryan N Kehoe, Robert T Dorsam, Soochong Kim, Satya P Kunapuli; Temple Univ Sch of Medicine, Philadelphia, PA

Injury to a blood vessel exposes subendothelial collagen and initiates the coagulation cascade promoting thrombosis. We hypothesized that hematopoietic signaling molecule downsteam from protease-activated receptor-1 (HS1), a 75 kDa tyrosine phosphorylated adapter protein, expressed in cells exclusively of hematopoietic lineage, is a critical regulator of platelet activation. Here we investigate the role of HS1 downstream of GPVI receptors in PAR receptor signaling pathway. We observed both a bleeding diathesis and an inhibition of thrombus formation by the in vivo FeCl3, thrombosis model, indicating HS1’s involvement in multiple pathways. HS1 phosphorylation occurs downstream of both PAR-1 and PAR-4, in a Gq dependent manner, however, ADP secretion has no effect on HS1 phosphorylation. HS1 is phosphorylated initially by Syk tyrosine kinase on tyrosine residues 397 and 378. Syk then dissociates from HS1, allowing for docking and subsequent phosphorylation of HS1 by Src family kinases on tyrosine residue 222. We demonstrate that Src family kinase inhibitors abolish the activation of Syk, as measured by its phosphorylation on tyrosine residues S255/256, and also completely block HS1 phosphorylation. This is in contrast to the previously reported results indicating that inhibition of Src family kinases results in partial HS1 phosphorylation, possibly on the Syk phosphorylated residues. We propose that Src is directly downstream of PAR receptors and phosphorylates Syk, which in turn phosphorylates HS1. Further, studies with HS1 null mouse platelets show an inhibition of aggregation, secretion and thromboxane A2 generation compared to their wild type littermates. HS1 null mice also display a decrease in AII and EriK phosphorylation, signaling molecules shown to be important in aggregation and thromboxane generation. Taken together with our previous results,
Bacterial lipopolysaccharide (LPS) activates Toll-like receptor 4 (TLR4) leading to rapid transcriptional activation and hypoxia-inducible factor-1α (HIF-1α) expression. Although evidence suggests that platelet activation plays important roles in LPS-induced thrombocytopenia and tissue damage, the effect of LPS on platelet activation and the signaling mechanisms of LPS-induced platelet activation have not been defined. Here we show that LPS significantly enhances platelet aggregation and secretion induced by low concentrations of platelet agonists. The enhancement of platelet aggregation and secretion by LPS is abrogated by an anti-TLR4 blocking antibody, and platelets express important molecular components of TLR4 signaling, CD14, MD2, and the adaptor protein MyD88, as well as TLR4, suggesting that the effect of LPS on platelet aggregation requires TLR4 pathway. LPS alone induces ATP release and P-selectin expression in human platelets and promotes FeC3-Injured carotid artery thrombus formation. Furthermore, the enhancement of platelet aggregation by LPS is inhibited in PKG II deficient platelets as well as specific PKG inhibitor-treated platelets. LPS induces significant increase of intracellular cGMP levels in platelets. Thus, our data indicate that LPS induces platelet secretion and promotes platelet aggregation and thrombosis by a TLR4 and PKG II dependent signaling pathway.

CD36 Modulates Platelet Reactivity in Vivo During Hyperlipidemia: A Mechanism Linking Hyperlipidemia, Oxidant Stress, and a Prothrombotic Phenotype

Eugene A Podrez, Maria Febbraio, Cleveland Clinic, Cleveland, OH; Robert G Salomon, Case Western Reserve Univ, Cleveland, OH; Yi Ma, Cleveland Clinic, Cleveland, OH; Eugenia Poliakos, Case Western Reserve Univ, Cleveland, OH; Manojkumar Valiyaveettil, Cleveland Clinic, Cleveland, OH; Mingjiang Sun, Case Western Reserve Univ, Cleveland, OH; Brian R Curtis, Blood Center of Wisconsin, Milwaukee, WI; Paula J Ferrit, Juhua Chen, Renliang Zhang, Roy L Silverstein, Tatiana V Byzova, Stanley L Hazen, Cleveland Clinic, Cleveland, OH

Enhanced platelet reactivity is critical to the pathophysiology of occlusive arterial thrombotic disease. Despite the strong clinical associations between hyperlipidemia, a major risk factor for atherosclerosis, and a pro-thrombotic phenotype, the mechanisms responsible for enhanced platelet reactivity during hyperlipidemia remain unknown. Pro-atherosclerotic lipid abnormalities such as hypercholesterolemia are associated with both enhanced oxidant stress and generation of biologically active oxidized lipids, including potential ligands for the scavenger receptor CD36, a major platelet surface glycoprotein. Using multiple murine in vivo models and hyperlipidemic atherosclerosis-prone apo E-deficient or LDL receptor-deficient mice, we now demonstrate that these mice form occlusive intrathrombi faster than wildtype mice, and that genetic deletion of CD36 protects mice from hyperlipidemia-associated enhanced platelet reactivity and accompanying pro-thrombotic phenotype. Structurally defined oxidized choline glycerophospholipid molecular species that serve as endogenous high affinity ligands for CD36 are shown to be increased in human plasma that markedly increases in plasma of hyperlipidemic mice and to promote platelet activation and alpha-granule release via CD36 at pathophysiological levels. These studies thus demonstrate that platelet CD36 interactions with specific endogenous oxidized lipids play a heretofore unrecognized role in the well-known clinical associations between hyperlipidemia, oxidant stress and a pro-thrombotic phenotype.

NOR1, an important transcriptional regulator of hepatic gluconeogenesis and inflammation, is expressed in vascular smooth muscle cells (SMC) of atherosclerotic lesions. NOR1 expression is increased in atherosclerotic lesions and promotes platelet aggregation and thrombosis by a TLR4 and PKG II dependent signaling pathway. Furthermore, the enhancement of platelet aggregation by LPS is inhibited in PKG II deficient platelets as well as specific PKG inhibitor-treated platelets. LPS induces significant increase of intracellular cGMP levels in platelets. Thus, our data indicate that LPS induces platelet secretion and promotes platelet aggregation and thrombosis by a TLR4 and PKG II dependent signaling pathway.

Role of the NR4A Nuclear Receptor NOR1 in Neointima Formation and Vascular Smooth Muscle Cell Proliferation

Takashi Nomiyama, Florence Gizzard, Elizabeth B Heywood, Karrie L Jones, Dennis Bruemmer; Univ of Kentucky, Lexington, KY

Nuclear hormone receptors comprise a large superfamily of ligand-activated transcription factors and have emerged as key regulators of glucose metabolism and inflammation in diabetes and cardiovascular diseases. The neuron-derived orphan receptor-1 (NOR1) belongs to the ligand-independent NR4A receptor subfamily which has previously been characterized as important transcriptional regulator of hepatic gluconeogenesis and inflammation. We have recently demonstrated NOR1 expression in vascular smooth muscle cells (SMC) of atherosclerotic lesions and outlined a key role for NOR1 to regulate SMC proliferation in vitro. In the present study, we demonstrate that SMC isolated from NOR1-deficient mice exhibit decreased cell proliferation due to a G2/M arrest of the cell cycle. NOR1-deficiency results in diminished phosphorylation of the retinoblastoma protein, cyclin D1 and D2 expression and mitogen-induced degradation of the cyclin-dependent kinase inhibitor p27. Using microarray technology we further characterize Sip1, C11, and Nedd6, key proteins of the ubiquitin ligase complex responsible for degradation of the proapoptotic protein p53. Finally, using a model of guide-wire induced arterial injury we observed decreased neointima formation in NOR1-deficient mice. These experiments demonstrate that NOR1 functions as a key transcriptional regulator of SMC proliferation and neointima formation by inducing cyclin D1 and the expression of genes required for the ubiquitination and degradation of p52. Therefore, NOR1 is a key regulator of SMC proliferation and may provide an important molecular target for the treatment of cardiovascular diseases.

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Regulation of Smooth Muscle Cell Proliferation by Hyaluronan and CD44

Devashish Kothapalli; Univ of Pennsylvania Sch of Medicine, Philadelphia, PA

High molecular weight hyaluronan (HWM-HA) is a widely distributed component of the ECM, but its biological activities remain incompletely understood. We previously reported that HWM-HA binding to CD44 antagonizes mitogen-induced S phase entry in cultured vascular smooth muscle cells. We now characterize the underlying molecular mechanisms and document its relevance during vascular injury in vivo. In particular, we show that HWM-HA inhibits the mitogen-dependent induction of cyclin D1 and degradation of p27kip1 in vascular smooth muscle cells. These effects were associated with an inhibition of Rb phosphorylation, cyclin A induction, and S phase entry. p27kip1 mRNA levels were unaffected by HWM-HA, but the expression of Skp2, the rate-limiting component of the SCF complex that degrades p27kip1, was reduced. Rescue experiments identified cyclin D1 as the primary target of HWM-HA. Similar effects were detected in fibroblasts. These effects were not detected in vascular smooth muscle cells isolated from CD44-null mice. Moreover, arteries from HWM-HA and CD44-null mice showed that the effects of HWM-HA/CD44 on cyclin D1 and Skp2 expression are detected in vivo and associated with altered smooth muscle cell proliferation after vascular injury. Our data indicate that HWM-HA is anti-mitogenic for multiple mesenchymal cell types and identify cyclin D1 as a major target of HWM-HA binding to CD44.

The CX3C Chemokine Fractalkine Acts via an Extracellular Regulated Kinase-Dependent Mechanism to Induce Human Coronary Artery Smooth Muscle Cell Proliferation

Gemma E White, Alison E John, David R Greaves; Univ of Oxford, Oxford, United Kingdom

Chemokines are important mediators of cell adhesion and migration that are expressed in both normal and pathological conditions and signal through G protein-coupled receptors. Fractalkine (CX3CL1) is an atypical membrane-bound chemokine that signals through its receptor CX3CR1 and has been implicated in the development and progression of atherosclerosis. We have previously reported that CX3CL1 is expressed by smooth muscle cells in human coronary artery atherosclerotic plaques and by primary human coronary artery smooth muscle cells (HCMC). We now report that CX3CL1 can also function as a mitogen for human HCMC in vitro. Experiments were replicated using cells from three independent donors and a HCMC line, HCM-601EB. Using three independent methods to measure cell proliferation, we now demonstrate that CX3CL1 induces primary human coronary artery smooth muscle cells to proliferate. Moreover, both the presence of CX3CL1 and its receptor CX3CR1 are required for this effect. This may have relevance in other vascular pathologies including restenosis and transplant arteriopathy.

Adipocyte-specific Low-density Lipoprotein Receptor–Related Protein-1 as a Novel Regulator of Adiposity, Energy Expenditure, and Glucose Metabolism

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Hypothalamic adipose tissue has been identified as an essential endocrine organ for the control of glucose homeostasis and energy balance. The multifunctional receptor LR-1 is expressed in adipose tissue where it mediates cellular cholesterol uptake. Herein we used the adipose tissue-specific LRP-1 knockout mouse model (ad-LRP1KO) to test the hypothesis that adipocyte LRP-1 plays an essential role in lipid storage and energy metabolism. Adipocyte-specific LRP-1 inactivation in mice (n=12) resulted in delayed adipose tissue lipid clearance (area under the curve [AUC]; ad-LRP1KO: 31925 +/- 6397 vs. wild type [WT]: 16512 +/- 1949, P < 0.05), lower body weights (BW: 22.4 +/- 1.4 vs. 28.3 +/- 0.8 g, P < 0.001), smaller fat stores (4.3 +/- 0.5 vs. 9.5 +/- 1.4% fat of BW, P < 0.002), lipid-depleted brown adipocytes, improved glucose tolerance (AUC; 20016 +/- 517 vs. 27251 +/- 1681, P = 0.05), elevated fatty acid oxidation, increased energy expenditure (+30 kcal/kg/d, P < 0.002), and increased food intake (1.5 +/- 0.2 vs. 1.1 +/- 0.1 g BW/d, P < 0.05). The slightly higher calorific intake may represent a compensatory mechanism. Intriguingly, such increased thermogenesis in ad-LRP1KO mice was confirmed by increased body temperature and paralleled by muscle shivering, quantified using a multi-dimensional light beam system to analyze motor activity (7102 +/- 439 in KO vs. 4013 +/- 333 WT beam strokes/3h, P < 0.001). Additional radiolabeled studies revealed that glucose and lipid uptake were significantly increased in skeletal muscle of ad-LRP1KO mice compared to WT mice (glucose: 12830 +/- 6515 vs. 8835 +/- 1203 pmol/mass, P < 0.01; oleic acid: 8824 +/- 662 vs. 4791 +/- 595 pmol/mass, P < 0.05), suggesting that skeletal muscle compensates for decreased thermogenic function of BAT in ad-LRP1KO mice, which may be reflected in the shivering phenotype. When placed for 4 weeks on high fat diet ad-LRP1KO mice were resistant to diet-induced obesity (11.2 +/- 1.5 vs. 22.2 +/- 2.7% % fat of BW, P < 0.002) and glucose intolerance (AUC: 23150 +/- 1295 vs. 30920 +/- 2074, P < 0.005). We conclude that reducing lipid transport to adipocytes and enhancing muscular energy expenditure via adipocyte LR-1 inhibition may be an efficient strategy to prevent diet-induced obesity and diabetes.

In Vivo Kinetic of C-Reactive Protein and Its Interaction with Features of the Metabolic Syndrome in Men and Women

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BACKGROUND Individuals with elevated (>3.0 mg/l) plasma C-reactive protein (CRP) levels, a key feature of the metabolic syndrome, are at greater risk for cardiovascular disease than individuals with low levels of CRP (<1.0 mg/l). The in vivo kinetics of CRP and the physiological mechanisms responsible for the observed sub-acute-phase circulating CRP levels in the metabolic syndrome and obesity are virtually unknown. Here we describe for the first time the intravascular kinetic of CRP and its relationship with features of the metabolic syndrome. METHODS Sixteen men and 16 women (aged 48-9 years, BMI = 28.7 +/- 4.9 kg/m2) underwent a 12-hour primed constant infusion of D3-L-leucine in the constant fed state. Blood samples were drawn at pre-determined time points. CRP was purified from the plasma fraction d:<1.25 g/ml at each time point by affinity chromatography followed by SDS-PAGE. Isotopic enrichment was determined by GC-MS. Plasma CRP levels were measured with high sensitivity using a commercial ELISA. RESULTS Mean CRP production rate (PR) and pool size (PS) were similar between men and women (0.030 +/- 0.026 vs. 0.032 +/- 0.039 mg/dl and 4.66 +/- 3.39 vs. 4.64 +/- 3.94 mg/l). However, the fractional cathodic CRP (FCR) of CRP in men was 60% higher than in women (0.57 +/- 0.29 vs. 0.35 +/- 0.20 pool/day, P<0.05). Circulating CRP concentrations were more strongly correlated with its FCR (r = -0.91, P = 0.0001) than its FCR (r = 0.55, P = 0.05). PR of CRP was directly correlated with BMI (r = -0.42, P = 0.02), waist girth (r = 0.44, P = 0.02), and with plasma LDL apoB-100 (r = 0.38, P = 0.05), triglyceride (r = -0.35, P = 0.05) and interelukine-6 levels (r = 0.50, P = 0.05). An inverse trend was also observed between the PR of CRP and HDL-C (r = -0.34, P = 0.06) while LDL-C and blood pressure showed no association with CRP kinetics. CONCLUSIONS Men were characterised by a 60% greater FCR of CRP compared with women. However, sub-acute-phase CRP levels appeared to be mainly explained by the PR of CRP rather than its FCR. Our results also suggest that features of the metabolic syndrome may be more predictive of CRP kinetics than traditional risk factors like LDL-C and hypertension.

Reduced Macrophage Infiltration in Visceral Adipose Tissue of 12-Lipoxygenase Knockout Mice

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Obesity induces the accumulation of macrophages in adipose tissue and generates a state of low-grade inflammation which is associated with the development of type 2 diabetes and release of pro-atherogenic cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Our recent studies indicate that 12-Lipoxygenase (12-LO) expression is increased in isolated visceral adipose models of insulin resistance and that deletion of 12-LO prevents the increases in TNF-α and IL-6 during high fat feeding. The aims of this study were to examine the role of 12-LO pathway in the accumulation of macrophages in adipose tissue of mice fed a high-fat diet. C57BL/6J (B6) and 12-LO knockout (12-LO KO) mice on the B6 background were fed chow diet for 4 weeks. At 12 weeks, mice were rapidly switched into a high-fat diet and were maintained in the visceral fat pad were examined by FACS and immunohistochemistry. Macrophage content in fat with chow feeding was similar in 12-LO KO and B6 mice. Western diet significantly increased macrophage content in fat tissue compared with chow diet in B6 mice. However, these mice are significantly reduced macrophage content in 12-LO KO mice compared to mice as shown in the table below. After the mice were fed Western diet for 24 weeks, the percentage of positive Mac2 staining within visceral fat pad was markedly increased in B6 mice compared to 12-LO KO mice (0.58 +/- 0.07 % vs. 0.26 +/- 0.03 % in male mice, respectively, P < 0.01; 0.32 +/- 0.04 % vs. 0.18 +/- 0.02 % in female mice, respectively, P < 0.01). These data suggest that 12-LO plays a key role in regulating macrophage trafficking and inflammation in visceral fat in states of obesity. Decreased localization of 12-LO knockout mice could provide a novel therapeutic approach to reduce the inflammatory state with visceral adiposity.
Fatty Acid Desaturase Gene Expression in Human Adipose Tissue Is Regulated by Dietary Composition Independent of Energy Restriction and Is Correlated with Plasma Triglyceride Response

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Atherogenic dyslipidemia, associated with elevated triglycerides and reduced HDL, is independently improved by dietary energy restriction and reduced carbohydrate consumption in an equivalent but non-additive manner. We assessed the hypothesis that total energy restriction and isocaloric alteration in dietary composition regulate common molecular pathways involved in lipid metabolism by monitoring transcriptional expression in human adipose tissue. Subcutaneous adipose biopsies were obtained from 131 moderately overweight, otherwise healthy men (BMI, 29.2 ± 2.0 kg/m²) following (1) one week on basal diet (54:15:30 [7], carbohydrate:protein:fat [saturated fat]), (2) three weeks on randomized diet differing in nutritional composition (basal, 39:29:31 [8], 26:29:46 [9] or 26:29:46 [15]), (3) five weeks of acute weight loss on randomized diet (−1103.0 ± 216.5 kcal/d resulting in −10.0 ± 3.3 lb), and (4) four weeks stabilized at reduced weight. Transcriptional responses were characterized using genome-wide expression array analysis on samples from thirteen subjects and findings for the most responsive genes were confirmed using real time PCR across all subjects. Energy restriction resulted in significantly reduced expression of 1473 transcripts and, of these, 30 were responsive to isocaloric alterations in dietary composition. Twelve of these genes are involved in energy metabolism including four in lipogenesis and five in lipid metabolism. Significant responses were confirmed for four top-changing genes (p < 0.003): stearoyl CoA desaturase (SCD), fatty acid desaturases 1 and 2 (FADS1, FADS2), and diacylglycerol transferase 2 (DGAT2). SCD response was strongly correlated with carbohydrate intake (p = 0.019) and, on a low carbohydrate diet, was inversely correlated with saturated fat intake (p < 0.05). Moreover, plasma triglyceride responses to changes in dietary composition were independently correlated with SCD (p = 0.003) and DGAT2 (p = 0.05) response. In conclusion, fatty acid desaturases in human adipose tissue are independently regulated by energy restriction and dietary composition and may be involved in dietary regulation of systemic triglyceride metabolism.

Effect of Protein, Monounsaturated Fat, and Carbohydrate Intake on Plasma Apolipoprotein B and VLDL and LDL Particles Containing apoCIII: Results from the OmniHeart Trial

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Plasma apoB and VLDL and LDL particles with apoCIII are independent risk factors for cardiovascular disease. We examined the effect of three healthy diets modeled after the DASH diet on these apolipoproteins and lipoproteins. All diets were high in fruits, vegetables, and whole grains and low in saturated fat, but differed by emphasis of either carbohydrate (CARB), monounsaturated fat (MONO), or protein (PROT). In the setting of a controlled, 3 period cross-over feeding study, healthy subjects, N = 164, consumed each diet for 6 weeks. As shown in the table below, all three diets similarly lowered plasma apoB and VLDL + LDL cholesterol compared to baseline when the participants ate their own diet. Only the PROT diet significantly lowered plasma triglyceride (TG). LDL particles without apoCIII (the major LDL type) were reduced equally by all diets, with an accompanying reduction in their TG and cholesterol concentrations. The PROT diet reduced LDL with apoCIII compared to baseline and CARB. In contrast, compared to baseline, CARB and MONO diets but not the PROT diet increased VLDL with apoCIII. In VLDL without apoCIII, the PROT diet reduced TG compared to baseline and CARB and cholesterol compared to baseline. The diets did not affect the molar ratios of TG to apoB and cholesterol to apoB in any of the particle type suggesting that the diets did not alter particle composition. In conclusion, substituting protein for carbohydrate reduced atherogenic apoCIII-containing LDL and had the most favorable effects on the VLDL particle types, resulting in the least atherogenic lipoprotein profile.

<table>
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<th>Change from Baseline</th>
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<tr>
<td>ApoB in plasma</td>
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<td>TG in plasma</td>
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<td>Chol in VLDL=LDL</td>
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<td>ApoB in LDL with no apoCIII</td>
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<td>ApoB in LDL with apoCIII</td>
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<td>ApoB in VLDL with apoCIII</td>
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<td>Chol in VLDL with no apoCIII</td>
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<td>ApoB in VLDL with apoCIII</td>
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<th>Between Diet Differences</th>
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<tr>
<td>ApoB in plasma</td>
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<td>Chol in VLDL=LDL</td>
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<td>Chol in VLDL with no apoCIII</td>
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<td>ApoB in VLDL with apoCIII</td>
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*p < 0.05, TG - triglyceride, chol - cholesterol
Factor XIII Levels Are Normal in Aflibrinogenemic Plasma

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Blood coagulation factor XIII (FXIII) is found in plasma and platelets. During the final stages of coagulation, FXIII catalyzes the cross-linking of adjacent fibrin monomers through the formation of γ-glutamyl-ε-lysyl isopeptide bonds. Prior studies have shown plasma FXIII is associated with fibrinogen, forming a non-covalent FXIII/fibrinogen complex. This finding suggests that FXIII levels in blood are dependent on circulating fibrinogen levels. To determine whether the absence of fibrinogen affects circulating FXIII, we measured FXIII levels in an aflibrinogenemic (fibrinogen-deficient) individual. We used gel electrophoresis and immunoblotted procedures to determine FXIII levels in plasma from an aflibrinogenemic individual and plasma pooled from normal individuals. We analyzed platelet-poor plasma in order to eliminate the cellular FXIII. Samples were prepared at fifty-, one-hundred-, and two-hundred- fold dilutions. Immunoblot analysis was performed using an affinity-purified, sheep anti-FXIII subunit A specific antibody. We found FXIII levels in the aflibrinogenemic and normal plasma samples were indistinguishable at all dilutions, indicating comparable physiological concentrations of FXIII in the fibrinogen-deficient and normal plasmas. These results show that the concentration of FXIII in plasma is independent of the presence or absence of fibrinogen. Furthermore, these data indicate FXIII can stably circulate in plasma even in the absence of fibrinogen and fibrinogen is not required as a carrier for circulating plasma FXIII.

Regulation of Factor Xa Mediated Signal Transduction by Annexin 2

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The serine protease zymogen factor X is converted to its catalytically active form factor Xa (Xa) by the binary complex of factor Va bound to its cell surface receptor tissue factor (TF) or alternatively by the intrinsic Xase complex which consists of active factors VIIa (VIIa), IXa (IXa), factor X, and Ca2+. Xa has procoagulant activity to convert prothrombin to thrombin and also induce cell signal transduction, either alone or in the ternary TF/VIIa/Xa complex. Xa cleaves and activates procoagulate activated receptor (PAR)1 or 2, but Xa signaling efficiency varies between cell-types. We now observe that Annexin 2 acts as a receptor for Xa on the surface of human endothelial cells (HUVEC) and that Annexin 2 association facilitates Xa activation of PAR-1, but not the coagulant function of Xa. Over-expression of TF abolishes Annexin 2-dependence of Xa signaling and diminishes binding to cell surface Annexin 2. We propose that Annexin 2 serves to regulate Xa cell signaling specifically in the absence of cell surface TF and may thus play physiological or pathological roles when Xa is generated by the intrinsic coagulation pathway.

Effect of Exercise Training on Endothelium-dependent Vasodilation in Aged Rat Is Associated with Reduced Caveolin Status

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Ageing impairs endothelial-dependent vasodilation in humans and animals. In the endothelium, caveolin-1 regulates nitric oxide signaling by binding to and inhibiting endothelial nitric oxide synthase(NOS). The purpose of this study was to examine whether exercise training alleviates impaired endothelial-dependent dilation of aorta in aged rats by 12 weeks of treadmill exercise and to determine their mechanisms. Three- and twenty-two-month-old male Fischer344 rats were assigned to young sedentary, young exercise-trained, old sedentary, or old exercise-trained groups. Abdominal aortic rings were prepared and vascular responses to acetycholine(10⁻⁹ – 10⁻⁷ M) were determined in vitro. To determine the potential role for nitric oxide and caveolin-1 in vasodilation in sedentary and exercised old rats, we examined serum activity of NOx and cGMP in aortas of old rats. Training improved the ageing-induced reduction in endothelium-dependent vasodilation in aortic preparations. Expression of eNOS RNA in aorta was unchanged by exercise training, whereas serum NOx level was increased by ~3 times, while caveolin-1 expression was decreased exaggerated by Western blot and immunostaining. We conclude that (1) exercise can improve impaired, endothelium-dependent dilation of aorta by ageing, (2) exercise can restore age-dependent loss of NO by altering eNOS subcellular distribution and its association with inhibitory proteins, caveolin-1, and (3) exercise may modify vascular reactivity in old subjects by altering levels of eNOS protein in the large artery. These results imply the design of clinical strategies that approach the age-associated loss of endothelial NO availability and through targeting these pathways.

Anemia Has Been Shown to Be an Adverse Indicator in Patients with Acute Coronary Syndrome and in Chronic Heart Failure

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Anemia is associated with an increased number of adverse cardiovascular events (CVD) in particular with coronary artery disease (CAD), and chronic heart failure (CHF), and it is also correlated with gender, aging, renal insufficiency, low BMI. Anemia involves inflammatory cytokines (C-reactive protein, IL-6), it reduces marrow response to erythropoietin (EPO) and heme-oxigenases-(HO-1), it also reduces red cells life span and it may impairs reuse of iron, it mostly reduces, the peak VO2 (peak aerobic power). The latter appears to be an independent factor that may be associated with an adverse outcome, in fact, for a reduction of one gram of hemoglobin (Hb) the risk of mortality and morbidity increase respectively by 22% and 18%. The aim of the study was to determine the clinical implication of anemia in patients with CHF or CAD: we have studied 38 patients (24 male, 14 female) with CHF, and 42 patients (28 male, 14 female) with CAD, with a range of Hb concentration included between 9.4g/dl and 12.6g/dl. We have evaluated moreover the tolerance to exercise on a treadmill and six minute walk distance (210–32 m in CAD, 190–32 m in HF), the presence of rest dyspnea, the presence of supraventricular or ventricular arrhythmias (atrial/or ventricular premature beats, sinus tachycardia, or ventricular tachycardia, atrial fibrilation); lower levels of Hb, Fe, TIBC correlate with a greater tendency to develop ventricular arrhythmias instead of supraventricular arrhythmias. Anemia management included erythropoietin stimulating protein , blood transfu- sion; we have used darbopoeitin 50 mcg every week, and this treatment is associated to a significant improvement in functional class and cardiac and renal function. Our data also confirm the link between an increased tendency to develop CVD and a decreased level of Hb.
NO donors and L-arginine than WT platelets. Low levels of exogenous NO (<100 nm) stimulate intraplatelet cGMP up to 4 fold and completely inhibit thrombin-induced aggregation of human platelets. These effects of NO on aggregation and cGMP levels are both reversed by added TRP (0.2 to 2 nm) or the recombinant C-terminal domain of TRP (2 nm) and CD47 against peptides (1 to 10 uM) derived from it. These results suggest that earlier attempts to assess the role of TRP in platelet aggregation did not adequately take into account the ephemeral nature of NO. In conclusion, TRP and CD47 have a discernable and significant role in promoting platelet aggregation, and perhaps stabilizing aggregates, under more physiological conditions of nitric oxide tone. (Supported by the National Institutes of Health.)

Predictors of Left Atrial Thrombus in Atrial Fibrillation

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Background: Stroke in atrial fibrillation (AF) encompasses mechanisms other than embolization. The CHADS-2 score is highly predictive of stroke in this setting yet its specificity for left atrial thromboembolism is not clear. Whereas warfarin is more effective for cardioembolic compared to non-cardioembolic stroke prevention, we sought to assess the correlation between left atrial thrombus (LAT) assessed by transesophageal echocardiography (TEE) and CHADS-2 score. Methods: Between 2000–2005, 179 cases with LAT (mean age 70; 12 years; 46% women) and 440 controls (71 years; 38% women) without LAT were identified. Clinical and echocardiographic variables are summarized (Table). The mean CHADS-2 score was low, but significantly higher for cases (average 2.4; 1.6; median 1.0; range 0 – 6) compared to controls (average 1.6; =: 1.3; median 1.0; range 0 – 6) (Figure). However, for those patients with TEE confirmed LAT, the CHADS-2 score varied considerably with 34% of CHADS-2 scores between 0 – 1. Furthermore, high scores (5 or 6) were uncommon (12.9%). Conclusions: The prevalence of LAT confirmed by TEE is not reliably predicted by the CHADS-2 score system. Left atrial size and left ventricular function remain the strongest risk variables for presence of LAT and may provide mechanistic insight into thrombogenesis in atrial fibrillation.

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Platelet Activity, Coagulation, and Fibrinolysis During Exercise in Healthy Males: Effects of Thrombin Inhibition by Argatroban and Enoxaparin

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Background—Relationships between exercise-induced activation of platelets, blood coagulation, and fibrinolysis, and the importance of thrombin for responses to exercise are not clear. Methods and Results—Effects of thrombin inhibition on haemostatic parameters were examined in a double-blind, cross-over study comparing the direct thrombin inhibitor argatroban (350 μg/kg i.v. bolus followed by 25 μg/kg/min infusion), the indirect thrombin inhibitor enoxaparin (0.75 mg/kg i.v. bolus), or placebo (saline) in 21 healthy males. Measurements were made at rest, before and during/after thrombin inhibitor treatment, and immediately after exhaustive exercise. At rest argatroban abolished, and enoxaparin attenuated platelet activation by thrombin, but not by ADP. Argatroban and, even more so, enoxaparin decreased thrombin generation (F1 +2) and the coagulation potential, and increased the fibrinolytic potential. Exercise increased circulating activated platelets from 5.5 ± 0.3 to 9.4 ± 0.9 x1010/μl (P < 0.001), circulating platelet-platelet microaggregates, the platelet responsiveness to in vitro stimulation, leukocyte activation (leukocyte CD11b expression and plasma elastase), and platelet-leukocyte aggregation (P < 0.01 for all). Exercise increased coagulation (F1 + 2; P < 0.01) and fibrinolysis, but did not alter the balance between them; fibrin gel permeability increased (P < 0.01). Neither argatroban nor enoxaparin counteracted exercise-induced platelet or leukocyte activation. Both thrombin inhibitors augmented exercise effects on fibrinolysis. Conclusions—Strenuous exercise enhances platelet and leukocyte activation independently of thrombin. Exercise augments both coagulation and fibrinolysis, but the balance between them appears to be maintained. At therapeutic dosages argatroban counteracted thrombin-induced platelet activation most efficiently, while enoxaparin had somewhat stronger antiocoagulant and pro-fibrinolytic effects.

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Regulated Expression of the Human Thrombin-activable Fibrinolytic Inhibitor Gene in Nonhepatic Cell Types

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The balance between coagulation and fibrinolysis is critical for the hemostatic response upon injury. The carboxypeptidase thrombin-activable fibrinolytic inhibitor (TAFI) plays a key role in controlling the balance by attenuating fibrinolysis; high plasma TAFI antigen levels have been associated with increased risk of thrombotic diseases. Activated TAFI (TAFIa) can also inactivate pro-inflammatory peptides such as the anaphylatoxins and bradykinin, suggesting a role for the TAFI pathway as a link between coagulation and inflammation. TAFI expression in HepG2 cells is decreased by interleukins -1 and -8 and plasma TAFI concentrations in humans are decreased in experimental endotoxemia. Although liver is presumably the main source of plasma TAFI, TAFI has also been identified in platelets, and TAFI mRNA has been detected in the Dami (megakaryoblastic) cell line, human umbilical vein endothelial cells, and adipocytes of patients with type 2 diabetes. Using RT-PCR and real-time RT-PCR, we now report the detection of TAFI mRNA in the human monocyctic cell line THP-1 as well as THP-1 cells that have been differentiated into macrophage-like cells (THP-1ma) by treatment with phorbol esters. We find no evidence of TAFI mRNA expression in human coronary artery smooth muscle cells. It has been hypothesized that platelet TAFI arises from TAFI gene expression in megakaryocytes. Accordingly, Dami cells were treated with phorbol esters for up to 72 hours to differentiate them along the megakaryocyte/platelet lineage. Using real-time RT-PCR analysis, we found that TAFI mRNA abundance was increased throughout Dami differentiation (up to 9-fold after 50 hours). Using real-time RT-PCR analysis, we also found that TAFI mRNA abundance was decreased when THP-1 and THP-1ma were treated with bacterial lipo polysaccharide for 24 hours (2-fold and 16-fold, respectively). Extra-hepatic expression of TAFI, such as in platelets, monocytes, and adipocytes, appears to have a significant role in localized TAFI expression in regulation of hemostasis and inflammation.

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Paclitaxel, but Not Zolatarolimus or Dexamethasamine, Inhibits Human Coronary Artery Endothelial Cell Serum-induced Migration in Vitro: Role of p70S6K Activation in Endothelial Cell Migration

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Introduction: Drug-eluting stents have reduced restenosis rates but elute agents which could interfere with endothelial cell (EC) migration and re-endothelialization from lesions, leading to thrombosis. Previous studies have shown that sirolimus blocks smooth muscle migration in response to individual growth factors such as PDKF-BB or sphingosine-1-phosphate an effect which may be mediated by inhibition of p70S6K activation. This study reports the effects of the anti-restenotic agents zolatarolimus, paclitaxel and dexamethasone on activation of p70S6K and its role in the migration of human coronary artery EC (HEC) induced by serum. Hypothesis: Paclitaxel but not zolatarolimus or dexamethasone is hypothesized to inhibit HEC migration. Reduced p70S6K activation alone will not result in anti-migratory activity. Methods: HEC migration in response to serum = growth factors was determined using a modified Boyden chamber. HEC were synchronized and treated for 0 or 24 hours in basal or growth media. After migration (24 hrs) cells were stained with calcein-AM and fluorescence measured. Phosphor p70S6K (T389) was measured by Western blot. Results and Conclusions: Paclitaxel but not zolatarolimus or dexamethasone blocks migration, however, all agents reduce p70S6K(T389) phosphorylation (figs). These data suggest that in the presence of multiple chemotactic factors, inhibition of p70S6K alone is insufficient to reduce migration. Dexamethasone and zolatarolimus should not impair, and the former may promote, re-endothelialization of vascular lesions. In contrast, paclitaxel is predicted to potentiate attenuate re-endothelialization by blocking HEC migration.

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WITHDRAWN
Background: The use of stents in current clinical practice has evolved to include complex lesions and high-risk patients who may be candidates for CABG. In such patients, DES is being used with increasing frequency. However, the comparative performance of DES and CABG is unknown. Methods: We examined in-hospital and long-term follow-up data from unselected patients who underwent isolated primary revascularization by DES or CABG at our institution. Two case-matched groups were created using the DES and CABG patient's propensity score. Early (30 day) major adverse cardiac events (MACE), including death, myocardial infarction, stroke and mid-term survival, using the Kaplan-Meier method, are reported. Outcomes were compared using Chi-Square and Log-rank statistics. Results: Of the patients who underwent revascularization, 1598 were identified with matched characteristics (DES, n= 799; CABG, n=799). Follow-up was available for three years. Early MACE was similar for both populations (DES 5.01% vs CAB 3.78%; p=NS). The risk of myocardial infarction and death were respectively similar (CABG vs DES; p=NS). However, stroke was more common in patients undergoing CABG (DES 0.11% vs CABG 1.11%; p<0.01). Estimated three-year mortality after revascularization was not different (CABG 6.8% vs DES 9%; p=NS). Conclusions: The data suggest that the risk of early MACE and late mortality after revascularization is similar for patients undergoing DES implantation or CABG.

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Comparison of Short- and Mid-term Outcomes of Patients with Coronary Artery Disease Treated with Drug-eluting Stents and Coronary Artery Bypass Grafting

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Hypothesis: Aspirin resistance remains rare when evaluated with the current gold standard (LTA-AA) in stable coronary artery disease patients. Platelet function tests are not equally effective in measuring aspirin’s antiplatelet effect and correlate poorly with one another, which may explain the discrepancies reported in the literature. Given the low agreement between the various assays, their usefulness to detect aspirin resistance is questionable.

PLATELET FUNCTION ASSAYS

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>Aspirin Resistance Prevalence</th>
<th>Coefficient of Agreement with LTA-AA</th>
<th>Coefficient of Agreement with LTA-AA</th>
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<tbody>
<tr>
<td>LTA-AA</td>
<td>4%</td>
<td>-</td>
<td>-</td>
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<tr>
<td>LTA-ADP 5 μM</td>
<td>10%</td>
<td>0.06</td>
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<td>LTA-ADP 10 μM</td>
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<td>LTA-ADP 20 μM</td>
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Impaired Endothelial Function in Human C-reactive Protein Transgenic Mice

Adrian Quan, Hwae Teoh, Sam Targari, St. Michael’s Hosp, Toronto, Canada; Alexander J Szalai, Univ of Alabama at Birmingham, Birmingham, AL; Michael E Ward, Suboth Verma; St. Michael’s Hosp, Toronto, Canada

BACKGROUND: Increasing evidence suggests that the inflammatory biomarker, CRP, may play a causal role in the development and progression of atherosclerosis. Since endothelial dysfunction is an early and integral component of atherosclerosis, we hypothesized that endothelial homeostasis would be impaired in vivo in human CRP transgenic mice. METHODS AND RESULTS: Male CRP transgenic (CRP Tg) and wild-type mice were injected (i.c.) thrice over two weeks with either peanut oil vehicle or turpentine to induce the inflammation-sensitive CRP transgene. Serum CRP was undetectable in wild-type mice, while levels in turpentine- and vehicle-treated CRP Tg were 272.28 ± 95.7 μg/mL and 1.41 ± 0.2 μg/mL (n=6–8, respectively). Endothelium-dependent and -independent vascular responses were studied using an isolated tissue bath technique. Aorta isolated from mice with elevated CRP levels demonstrated profound impairment in endothelium-dependent responses to acetylcholine (57.1 ± 9.5 vs. 65.9 ± 5.0, p<0.05, n=6), with no in vivo or ex vivo difference in endothelium-independent vasoactivity to SNP. Murine’s thromboxane staining revealed increased perivascular fibrosis in turpentine-treated CRP Tg mice compared to vehicle-treated CRP Tg mice. Furthermore, CRP overexpression in-vivo promoted an increased expression of MCP-1 and macrophage infiltration in mouse aortic tissue. CONCLUSIONS: We demonstrate that human CRP transgenic mice exhibit endothelial dysfunction in-vivo with resultant changes in vascular structure and endothelial resistance to injury. These data strengthen the role of CRP as a partaker of vascular risk.
ADP-stimulated platelets were perfused through a perfusion flow-chamber over a fibrinogen-induced platelet aggregation was rapidly inhibited by single-dose administration of AZD6140. ADP was significantly reduced 0.5–4 hours after single administration of AZD6140. ADP-inhibition is rapid and persistent. 

**Background**

Excessive platelet activation fundamentally contributes to cardiovascular events and mortality in patients with diabetes mellitus. We investigated whether acute administration of AZD6140, a reversible oral P2Y 12 antagonist with rapid onset of action, would beneficially modulate platelet reactivity in diabetic rats.

**Methods**

Streptozotocin-induced Diabetes

*Streptozotocin (30 mg/kg; i.v.) was administered to male Wistar rats. After 4 weeks of diabetes, platelet aggregation induced by ADP and collagen was measured in a platelet aggregometer.*

**Results**

Platelet aggregation induced by ADP and collagen was significantly inhibited by AZD6140 (10 μmol/L) in diabetic rats. In addition, ADP-stimulated platelet aggregation was inhibited by AZD6140 in diabetic rats. These findings suggest that AZD6140 effectively modulates platelet reactivity in diabetic rats.

**Conclusions**

AZD6140 may be a potential therapeutic target for vascular diseases associated with endothelial apoptosis.

**Localization of Coagulation Proteins Within the Arterial Vessel Wall in Relation to Progression of Atherosclerosis**

Henri M H Sprock, Sylvia Heeneman, Peter Kassak, Kargy Hamulyak, Mathew JAP Daemen, Hugo ten Cate; Univ of Maastricht, Maastricht, The Netherlands

**Introduction**

Thrombogenicity of the atherosclerotic plaque depends on, the quantity and activity of tissue factor localized in the arterial vessel wall. Recent experiments indicate that in addition to local tissue factor (TF), also factor (F) VII is expressed by cells in human atherosclerotic plaques. Hypothesis: Vitamin K dependent proteins including prothrombin and FV, are synthesized by cells within the arterial vessel wall and contribute to the thrombogenicity of atherosclerotic plaques. Methods: Immunohistochemical staining of TF and coagulation factor II (prothrombin) and factor VII (Hageman Factor) in atherosclerotic plaques and in the adjacent intima and media. Localization of TF was also performed in the swine myocardium. Firat factors IX and XI were found to be present in the coronary artery. We measured intramyocardial T in the area at baseline and after 20 to 30 minutes. 

**Results**

**Localisation of Coagulation Proteins**

- **TF was observed in both RCA occlusions with a mean ΔT of 0.25 ± 0.2°C.**
- **Time to onset of T changes was 23.7 ± 6.5 s, whereas EKG changes were apparent after 81.7 ± 12.3 s.**
- **Changes in T significantly preceded EKG changes of ischemia by 58 seconds (p < 0.01).**
- **Time to peak drop in T was 21.39 ± 10 s.**
- **EKGs and T changes began reverting to pre-ischemic baseline levels after 20 min.**

**Conclusion**

TF activity in atherosclerotic lesions appears to produce a detectable T change in the swine myocardium. T may be used as a novel and surrogate marker for myocardial ischemia.
the presence of metabolic syndrome criteria. Methods: 118 patients were evaluated retrospectively. The metabolic syndrome criteria including the presence of three of the five parameters were considered in the division of groups. While group 1 had metabolic syndrome (MS+), group 2 had no metabolic syndrome (MS−). All patients had blood withdrawn for GMT, other hematologic and lipid parameters, and blood pressure. Patients were diagnosed as MS+ if they had 3 of the following 5 abnormalities: (1) abnormal girth in men >102 cm, in women >88 cm; (2) triglycerides ≥150 mg/dL; (3) high-density lipoprotein (HDL) cholesterol in men <40 mg/dL or in women <50 mg/dL; (4) fasting blood glucose ≥110 mg/dL; (5) systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, or treated hypertension. The data compared includes; age, sex, body mass index, cigarette smoking and metabolic syndrome criteria.

Results: The demographic data including age and sex were not different in both groups. The serum GMT levels rise more dramatically in patients with MS (group 1; 26 ± 5.6 mg/mL) than patients without MS (group 2; 21 ± 2.2 U/mL, p < 0.05). The serum uric acid other hepatic enzymes, creatinine, and lipid levels did not show significant changes between groups.

Conclusions: Our study demonstrated that elevated serum GMT levels are closely associated with the presence of metabolic syndrome in patients undergoing coronary artery bypass graft surgery. GMT can be a diagnostic marker for the presence of coronary atherosclerosis and metabolic syndrome.

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High Glucose Augments Interferon-γ-Stimulated Matrix Metalloproteinase-1 Expression in U937 Macrophages by Enhancing STAT-1 Activity

Alena Nareika, Med Univ of South Carolina, Charleston, SC, Bryan A Game, Ralph H Johnson VA Med Ctr, Charleston, SC, Maria F Lopez-Virella, Yan Huang, Ralph H Johnson VA Med Ctr and Med Univ of South Carolina, Charleston, SC

Plaque rupture is a principal cause of luminal thrombosis in acute coronary syndromes occurring in 75% of patients who die of acute myocardial infarction. Recent studies called Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Research (DCCT/EDIC) have shown that rigorous glycemic in diabetic patients leads to a significant reduction of cardiovascular events, suggesting that hyperglycemia may contribute to destabilization of atherosclerotic plaques. Although mechanisms such as protein glycosylation by which hyperglycemia promotes atherosclerosis have been established, it is not well understood how hyperglycemia interacts with inflammatory cytokines such as interferon (IFN) gamma, a key cytokine involved in atherosclerosis, in inflammation-related plaque destabilization. In this study, U937 macrophages were cultured in medium containing either normal (2.5 mM) or high glucose (25 mM) were treated with 100 units/ml of IFN gamma for 24 h. After treatment, matrix metalloproteinase (MMP)-1 expression was examined. Results from real-time PCR showed that high glucose and IFN gamma increased MMP-1 mRNA level by 100% and 150%, respectively, whereas the combination of high glucose and IFN gamma resulted in an increased mRNA expression, suggesting a synergistic effect between high glucose and IFN gamma. Data from ELISA also demonstrated the similar synergistic effect on MMP-1 secretion. Besides MMP-1, high glucose and IFN gamma also synergistically stimulate MMP-9 and IL-1 beta, but had no effect on tissue inhibitor of metalloproteinase (TIMP)-1 and -2. Furthermore, the molecular mechanism underlying the synergistic effect was explored. Results from Western blot and electrophoretic mobility shift assay (EMSA) showed that high glucose augmented IFN gamma-stimulated MMP-1 expression by increasing phosphorylation and DNA-binding activity of signal transducing and activator of transcription (STAT)-1, a major transcription factor involved in IFN gamma-regulated gene expression. Given that hyperglycemia is a major abnormality in diabetes and diabetic patients have increased cardiovascular events, this study revealed a novel mechanism involved in diabetes-promoted cardiovascular disease.

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Effect of Chronic Insulin Plus Atorvastatin Therapy on Mitochondrial Function in an Ex Vivo Animal Model of Diabetes and Hypercaloric Diet Submitted to Global Myocardial Ischemia-Reperfusion

Pedro Monteiro, Marta Paiva, Raquel Carreira, Lino Goncalves, Luis A Providência; Coimbra Univ Hosp, Coimbra, Portugal

Introduction: Obese diabetics have more ischemic heart disease; this may be improved by insulin (INS) and atorvastatin (ATV). If these subjects are treated with INS or ATV and if therapy with INS improves mitochondrial function. Aim: To evaluate, in a model of diabetes and diet-induced hyperlipidemia in rats, whether insulin and/or atorvastatin improve mitochondrial function.

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Advanced Lipoxidation End Products Induce the Expression of Proinflammatory Cytokines and Chemokines in Human THP-1 Monocytes

Narkunanja Shanimugam, James L Figarola, Yan Li, Piotr M Swiderski, Samuel Rahbar, Rama Natarajan; Beckman Rsch Institute of City of Hope, Duarte, CA

Reactions of carbohydrates or lipid-derived intermediates with proteins lead to advanced glycation or -advanced lipoxidation end products respectively (AGEs/ALEs). AGEs/ALEs levels increase with age and in diseases like diabetes and atherosclerosis. Although the cellular effects of AGEs have been extensively studied, ALE effects have not yet been reported. In this study, we tested our hypothesis that ALE (malondialdehyde-lysine, MDA-Lys) can induce the expression of proinflammatory cytokines and chemokines in human monocytes in monocytes. We first adopted a profiling method using cytokine antibody arrays containing antibodies to 120 pro-inflammatory cytokines and chemokines. In the next approach, RT-PCR and qPCR analyses of total RNA showed that, similar to AGEs, MDA-Lys also significantly increased the mRNA expression of receptor for AGEs (RAGE) as well as Interferon-g-inducible protein 10, GRO, CXCL10, CXCL11, CXCL2, CXCL3, CXCL5, CXCL9, CXCL12 and CXCL13 in human monocytes. THP-1 monocytes were cultured either under normal glucose conditions (NG, 5.5 mM) or treated with in synthesized ALE ( MDA, Lys, 10ug/ml) or MDA alone (0.1nm-1 uM). After treatment, matrix metalloproteinase (MMP)-1 expression was examined. Results from Western blot and electrophoretic mobility shift assay (EMSA) showed that high glucose augmented IFN gamma-stimulated MMP-1 expression by increasing phosphorylation and DNA-binding activity of signal transducing and activator of transcription (STAT)-1, a major transcription factor involved in IFN gamma-regulated gene expression. Given that hyperglycemia is a major abnormality in diabetes and diabetic patients have increased cardiovascular events, this study revealed a novel mechanism involved in diabetes-promoted cardiovascular disease.
Dietary Cholesterol Selectively Increases Visceral Adipose Tissue Macrophage Accumulation in Obese LDL Receptor-Deficient Mice

Savitha Subramanian, Elzabeth A Kirk, Kevin D O’Brien, Alan Chait; Univ of Washington, Seattle, WA

In obesity, macrophages accumulate in adipose tissue and contribute to insulin resistance. Obesity also results in chronic low-grade systemic inflammation that is associated with insulin resistance, type 2 diabetes and contributes to atherosclerosis. We previously showed that dietary cholesterol induces low-grade inflammation and increased atherosclerosis in LDL-/– mice fed a Western diet. To study the interaction of dietary cholesterol and obesity on chronic inflammation, LDL-/– mice were fed either a rodent chow diet (control) or a diabetogenic diet (high in fat and CHO), without or with 0.15% cholesterol (C–C & D–C respectively). Weight gain was similar in D–C and D–C mice, and significantly more than controls. Insulin levels were higher and responsiveness to insulin was lower in the D–C as compared to the D–C group, suggesting an additive effect of dietary cholesterol on insulin resistance. As compared to controls, circulating levels of the inflammatory protein, serum amyloid A, were 2-fold higher in D–C animals and 5-fold higher in D–C animals, suggesting chronic inflammation. Morphometric analysis of epididymal (visceral) adipose tissue (AT) showed adipocyte hypertrophy and greatest macrophage inflammation in the stroma in the D–C group compared to the chow and D–C groups (P < 0.001). The D–C group also showed the greatest stromal expansion and proliferation of adipocyte as compared to chow-fed mice (P < 0.01). Visceral AT mRNA expression of the chemokine, MCP-1, and of the macrophage marker, F4/80, were highest in the D–C group (P < 0.05 & < 0.001 respectively). As compared to chow-fed animals, visceral AT mRNA levels were increased for TNF-α and decreased for adiponectin in both D–C and D–C diet-fed animals (both P < 0.05). While adipocyte hypertrophy was noted in subcutaneous AT, no significant macrophage accumulation was seen in any of the groups, and there were no differences between groups in adiponectin, F4/80 or TNF-α mRNA levels. These findings indicate that dietary cholesterol induces a chronically inflamed state, with increased macrophage infiltration and TNF-α production contributed to visceral, but not subcutaneous AT. The exact mechanism whereby dietary cholesterol leads to macrophage recruitment into visceral AT in obesity needs further elucidation.

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Effect of Increased Consumption of Whole Grain Foods on Markers of Cardiovascular Disease Risk in Middle-aged Healthy Volunteers

Paula Tighe, Nicholas Vaughan, Julie Brittenlend, William G Simpson, William Mutch, Univ of Aberdeen, Aberdeen, United Kingdom; Graham Horrigan, Garry Duthie, Rowett Rsch Institute, Aberdeen, United Kingdom

Epidemiological studies suggest that high intakes of whole grain foods may lower cardiovascular disease (CVD) risk. However, current recommendations that consumption of three servings of whole grain foods daily may be cardio-protective have not been validated. The aim of this on going study is to assess the effects of increased consumption of whole grain foods on markers of CVD risk in relatively high-risk individuals. Volunteers (n = 65; 40–65y, 38 males and 27 females), recruited from the local community, were randomised into one of three dietary intervention groups and asked to consume three portions per day of either refined, wheat-based or oat dietary intervention groups and asked to consume three portions per day of either refined, wheat-based or oat dietary intervention groups. Participants were randomised into one of three dietary intervention groups and asked to consume three portions per day of either refined, wheat-based or oat dietary intervention groups. Blood samples were collected at baseline (BL) and after 8 weeks. Blood samples were collected and analysed for lipid profiles, lipoproteins and high-sensitivity C-reactive protein. Blood pressure, body mass index (BMI) and arterial stiffness (determined by pulse wave velocity analysis) were also assessed at both the baseline and 8-week markers and assessed for differences significantly after dietary intervention. Arterial stiffness was also unaffected by dietary treatment. However, after adjustment for age, sex and BMI, both systolic and diastolic blood pressure decreased significantly in the wheat-based and oat + wheat-based groups compared to the refined group (table). This initial data indicates that the daily consumption of 3 portions of either wheat-based or oat + wheat-based whole grain foods may protect against CVD via a blood pressure-lowering mechanism.

Table

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Significantly lower for oat + wheat-based intervention compared to the chow and D–C groups (P < 0.001). Post-intervention HDL values were 141 (±14) mg/dL vs pre-intervention values of 127 (±2.6) mg/dL. Post-intervention ATP values were 40 (±14) mg/dL vs pre-intervention values of 77 (±1.3) mg/dL. Post-intervention HbA1c values were 7.2 (±1.0) mg/dL vs pre-intervention values of 9.2 (±1.0) mg/dL.

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Lipid and Glucose Optimization Using Phytomiton Combination Therapy in Diabetes

Peter J Verdegem, Unicity International, Orem, UT; Bobi Home, Isabel Martinez; Blue Mesa Med Associates, Katy, TX

Introduction: Dietary approaches to management of lipid and glucose parameters in diabetes is gaining popularity among patients. Monotherapy with dietary interventions has shown positive effects, but with limited clinical relevance. Our research focuses on using a polypharmacological combination in optimizing lipid and glucose parameters. All four ingredients have individual data supporting their use for optimizing lipoprotein fractions in hypercholesterolemia. This pilot study evaluates their combined efficacy in type-2 diabetes. Methods: A group of 34 subjects with established type-2 diabetes and hypercholesterolemia added the product to their diet. The drink was taken twice daily 15–20 minutes before meals. The fiber drink consists of viscous soluble fiber, minerals, vitamins, policosanol, phytosterols, and an aqueous Chrysanthemum morifolium extract. Lipid and glucose parameters were measured at baseline, 4 and 8 weeks.

Results:

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Impact of Omega-6 Polysaturated Fatty Acid:Eicosa pentanoic acid + Docosahexaenoic Acid Ratios in LDL-receptor Knockout (LDLr-/–) Mice on Atherosclerotic Lesion Formation and Elicited Peritoneal Macrophages Inflammatory Response

Shu Wang, Dayong Wu, Nirupa R Matthan, Alice H Lichtenstein; Tufts Universtiy, Boston, MA

Objective—Very long chain omega-3 fatty acids have been associated with decreased risk of CVD. LDL receptor knockout mice were used to assess the effect of different omega-6:EPA ratios on atherosclerosis lesion formation and elicited peritoneal macrophage inflammatory response. Methods and Results—Mice (n = 10/group) were fedathermic diets for 32 weeks: high fat (20% w/w) without EPA + DHA (HF–omega-6), and high fat with omega-6:EPA + DHA ratios of 20:1 (HF R20:1), 4:1 (HF R4:1), and 1:1 (HF R1:1). Aortic total cholesterol was 2.6%, 19.8% and 24.3% lower in the HF R20:1, HF R4:1 and HF R1:1 fed mice, respectively, compared with HF omega-6 fed mice. Elicited peritoneal macrophage cholesteryl ester content (mg/100mg protein) was 4.3 ± 1.3, 3.9 ± 1.1*, 2.6 ± 0.7 and 2.7 ± 0.7 values with different superscripts are significantly different at P < 0.05) in mice fed HF omega-6, HF-R20:1, HF R4:1, and HF R1:1 diets, respectively. Peritoneal macrophage membrane fatty acid profile reflected dietary treatment. Elicited peritoneal macrophages isolated from these mice were stimulated with lipopolysaccharide. Monocyte chemotactic protein-1 (MCP-1) release into the culture medium was 28 ± 7, 23 ± 9, 18 ± 8 and 17 ± 6 mg/mg protein in HF omega-6, HF-R20:1, HF R4:1, and HF R1:1 diet groups, respectively. Gene expression levels of MCP-1, tumor necrosis factor alpha (TNF-α), and ATP transporter cassette A1 (ABCA1) were significantly lower in elicited peritoneal macrophages from mice fed HF R1:1 compared to mice fed HF omega-6 diet. Conclusions—These data suggest that diets with lower omega-6:EPA DHA ratios resulted in a lower inflammatory response which was associated with less aortic lesion formation.

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CD36 Modulates Macrophage Spreading and Migration in Response to Oxidized Low-density Lipoprotein

Young M Park, Case Western Reserve Univ;Lerner Rch Institute, The Cleveland Clinic Foundation, Cleveland, OH; 18 Febraio, Roy L Silverstein; Lerner Rch Institute, The Cleveland Clinic Foundation, Cleveland, OH

CD36 is a class B scavenger receptor expressed on mononuclear phagocytes that has been implicated in many biological processes including atherosclerosis and angiogenesis. Previous work showed that CD36 binds and mediates uptake of oxidized LDL (oxLDL) and also transmits intracellular signals through src and MAP kinases. We hypothesized that oxLDL may affect cellular adhesion and migration via CD36-dependent signalling by modulating cytoskeletal function. Using a modified Boyden chamber migration assay, we showed that CD36 null mice did not native LDL induced random and MCP-1 induced mouse peritoneal macrophage migration, an effect abrogated in macrophages from CD36 null mice. OxLDL but not native LDL induced rapid spreading on serum-coated glass, and this was delayed in CD36 null cells. There were 2.7 fold more spread wild type than CD36 null cells 5 minutes after stimulation with oxLDL. This was also evaluated by measuring cellular area; wild type macrophages had 2 fold greater mean cellular area compared with CD36 null macrophages at this same time point. Western blots of extracts from macrophages incubated with oxLDL showed sustained phosphorylation (activation) of focal adhesion kinase (FAK) and dephosphorylation (inactivation) of src homology 2-containing phosphotyrosine phosphatase 1 (SHP-2). Inhibition of SHP-2 in oxLDL-treated cells was associated with oxidative modification of cysteine residues, detected by measuring 5-iodoacetamidofluorescein binding. Flow cytometry of macrophages incubated with fluorescently labeled phaloidin showed that oxLDL induced actin polymerization. These responses to oxLDL including activation of FAK, inactivation of SHP-2 and promotion of actin polymerization, were significantly blunted in macrophages from CD36 null mice. We conclude that CD36 signaling in response to oxLDL contributes to cytoskeletal rearrangement and modulates macrophage spreading and migration, and suggest that cytoskeletal rearrangements mediated by CD36 may induce trapping of macrophages in the arterial intima and thus promote atherosclerotic lesion development.
Sphingosine-1-Phosphate Induces an Anti-inflammatory Phenotype in Macrophages During Inflammation

Jennifer Hughes, Suseela Sinivasan, Catherine Hedrick; Univ of Virginia, Charlottesville, VA

Activated macrophages acquire a pro-inflammatory (classical) or anti-inflammatory (alternative) phenotype that influences atherosclerosis. Sphingosine-1-phosphate (S1P), a novel anti-inflammatory sphingolipid, serves a protective role in inflammation by changing the macrophage phenotype from a pro-inflammatory M1-classical phenotype to an anti-inflammatory M2-alternative phenotype. This study examined the anti-inflammatory effects of S1P on LPS-stimulated cytokine secretion in primary C57BL/6J (B6) mouse peritoneal macrophages. LPS upregulated mRNA expression of the pro-inflammatory cytokines TNF-alpha and MCP-1 by several-fold. Incubation of macrophages with S1P reduced TNF-alpha mRNA by 3-fold (p < 0.01) and MCP-1 by 2-fold (p < 0.001). MCP-1 decreased with TNF-alpha and MCP-1 as well. S1P increased TNF-alpha and MCP-1 secretion by LPS-stimulated macrophages by approximately 50%. Alternative macrophage activation is characterized by induction of the anti-inflammatory Th2 cytokine IL-10 and arginase-1. S1P triggered a 10-fold increase in IL-10 mRNA expression (p < 0.001) and a 2-fold increase in arginase-1 mRNA levels (p < 0.001). Arginase-1 enzyme activity was increased by 33%. Macrophages were activated by LPS alone to express inducible nitric oxide synthase (iNOS), and the response was significantly decreased by approximately 50% with the addition of S1P. Mouse peritoneal macrophages express S1P1 and S1P2 receptors. The uncoupling of S1P1 Gi signaling by the Gi1000 inhibitor pertussis toxin blocked the ability of S1P to reduce TNF-alpha and MCP-1 mRNA expression in LPS-stimulated macrophages. Treatment of B6 macrophages with the selective S1P2 receptor antagonist JTE-013 did not affect the ability of S1P to reduce LPS-mediated cytokine expression. Therefore, these results suggest that S1P1 is responsible, at least in part, for the alternative activation of macrophages during inflammation. We therefore conclude that S1P promotes the alternative anti-inflammatory macrophage phenotype through action on S1P1.

Hearts Require Lipoprotein-derived Fatty Acids (FAs) to Compensate for Chronic Angiotensin II Treatment

Haruyo Yamashita, Shota Ikeda, Tae-Sik Park, Ira Goldberg; Columbia Univ Med Cntr, New York, NY

Under metabolic conditions found in diabetes and metabolic syndrome, hearts are much more reliant on more cardiac energy from FA oxidation. Although such cardiac metabolic changes are thought to play a role in pathogenesis of reduced cardiac function, it is also likely that reduced glucose uptake is involved. Therefore, we examined the role of increased glucose uptake on the regulation of FA flux in hearts. In wild-type mice, we measured a 4-fold increase in glucose uptake (p < 0.001) and in low density lipoprotein (LDL) (3.3 ± 0.08 mg/dL) compared to the control. In heart, lipid uptake was increased in the treatment group (p < 0.001) and in LDL (3.3 ± 0.08 vs 2.9 ± 0.08 mg/dL; p < 0.001) compared to the control group. Therefore, these results suggest that the increased glucose uptake is not sufficient for hearts to compensate for hypertensive stress and that cardiac acquisition of LPL-derived FA has a critical role to allow normal heart function.

The Role of Different Pathways in the Release of Cholesterol from Foam Cells

Maria Pia Adoni, Francesca Zimetti, George H Rothblat; Children’s Hosp of Philadelphia, Philadelphia, PA

Introduction: Cholesterol efflux from cells occurs by different pathways, including aqueous diffusion, transport mediated by specific proteins, such as SR-BI, ABCA1 and ABCG1. The level of expression of these proteins is regulated by a number of different factors among which is intracellular cholesterol content. Previous observation made in Fu5AH hepatoma cells showed that the fractional (% efflux) of cholesterol to 15 human sera was similar with normal and enriched cells, suggesting that it was the same mechanism drive efflux, independent of cholesterol content. In contrast, mouse peritoneal macrophages (MMP) increased in fractional release of cholesterol to serum when cells were enriched by exposure to acLDL. Aim of study: The aim was to quantify the contribution of different efflux pathways using cholesterol-normal and enriched MMP exposed to human serum. This was accomplished by using MMP from ABCA1 and SR-BI KO mice and 2) using the specific ABCA1 and SR-BI inhibitors Protocoll and B/L-1. Methods: MMP were labeled with 3H-cholesterol with or without 50g/mg of acLDL. After an equilibration period, the cells were incubated in 2.5% human serum and the efflux of cholesterol was measured after 9h. The inhibitors Protocoll and B/L-1 were added at the beginning of the efflux phase. The initial cell cholesterol was measured by a fluorometric assay. Results: In WT MMP loading with cholesterol caused an increase in efflux by 90~100%. The contribution of SR-BI to the total efflux, measured by B/L-1 inhibition, was very low (1.7~3.2%) in both cholesterol normal and enriched cells. Consistent with this observation, cholesterol enrichment of SR-BI KO macrophages induced an increase in fractional release of cholesterol similar to that observed in WT cells. The ABCA1 contribution, as measured by Protocol inhibition of efflux, increased after cholesterol loading (from 2.4% to 8.5%). When ABCA1 KO cells were enriched there was no increase in the fractional release of cholesterol. Conclusion: In conclusion, our results demonstrate that the ABCA1 protein plays the major role in the stimulation of the fractional release of cholesterol upon enrichment of MMP. Additional experiments are being conducted to assess the role of ABCG1 in this process.

Acyl-CoA:Cholesterol Acyltransferase 2 and ATP-binding Cassette Half Transporters G5 and G8 Differentially Alter Hepatic Lipoprotein Secretion and Composition

Heather M Alger, Wake Forest Univ, Winston-Salem, NC; Paolo Parini, Karolinska Institutet at Huddinge Univ Hosp, Huddinge, Sweden; Ramesh Shah, Lifeng Yu, Lawrence L Rudel; Wake Forest Univ, Winston-Salem, NC

Three proteins that function in the regulation of cholesterol metabolism and are known to be expressed primarily in hepatocytes and enterocytes are: ATP-binding cassette half transporters G5 and G8 (G5G8) and acyl-CoA:cholyl ester acyltransferase 2 (ACAT2). G5G8 is responsible for inside-out transport of cholesterol in cells and ACAT2 is an endoplasmic reticulum-bound enzyme that esterifies cholesterol with acyl-CoA. One of the common processes that these proteins appear to regulate in both cell types is lipoprotein particle secretion. To identify the nature of this regulation, mice bearing gene deletions for G5G8 and/or ACAT2 were created. G5G8 KO mice had a higher liver-to-body weight ratios than wild type mice, apparently due to hepatic triglyceride (TG) accumulation. ACAT2 KO mice had lower hepatic TG accumulation but higher TG secretion. The double KO mice had hepatic triglyceride secretion rates and accumulation similar to that seen in ACAT2 KO mice. The data suggest that sterol handling regulates VLDL secretion and composition. Absence of cholesterol esterification resulted in increased VLDL particle secretion and decreased intracellular triglyceride accumulation while absence of cholesterol transport out of the cell via G5G8 resulted in triglyceride accumulation in the cell and decreased VLDL secretion. Thus, the pools of cholesterol accessed by G5G8 and ACAT2 appear to be different and impose separate outcomes on VLDL particle secretion vs. intracellular triglyceride accumulation. These effects were effectively balanced out in the double KO animals.
cholesterol and plasma TG, and FFA. The BALB/cByJ-apoB
strain had the largest increase in plasma TG of any strain, being 38.9mice showed significant insulin resistance compared to the wild-type group, the mutant group had a higher rate of diabetes mellitus, lower prevalence of drinking and alcohol consumption. The mutant group also showed a reduction in the coronary diameter and blood flow in response to sublingual administration of nitroglycerin, but the two groups were not statistically different in any other parameter. These results suggest that TRP12 and PHE15 are important for the function of apoA-IV in vivo.

**P117 Acetaldehyde Dehydrogenase-2 Gene Polymorphism in Han Chinese People with Acute Coronary Syndrome**

Feng Xu, Yu-guo Chen, Qiu Hosp of Shandong Univ, Jinan, China; Madi Bidya Sagar, Texas Heart Institute and Univ of Texas-Houston, Houston, TX; He Zhang, Chuan-xiao Jiang, Yun Zhang, Qiu Hosp of Shandong Univ, Jinan, China; Yong-jian Geng, Texas Heart Institute and Univ of Texas-Houston, Houston, TX

**Objective:** Acetaldehyde dehydrogenase-2 (ALDH2) gene polymorphism occurs in a significant number of Asians, leading to an altered metabolism of alcohol, other aldehydes or certain drugs. In this study we investigated the potential association of the ALDH2 gene polymorphism with acute coronary events, such as acute myocardial infarction (MI). In Han Chinese. **Methods:** DNA was isolated and analyzed by PCR-direct sequencing from peripheral blood samples of 271 Han people with coronary atherosclerotic heart disease (CAD). Coronary function was determined by coronary angiographic and ultrasound assessment of brachial artery dilatation. The severity of coronary atherosclerosis was evaluated by quantitative angiography with QCA analysis. **Results:** DNA sequencing identified the missense mutation (1/2→2/2) in 98 individuals. The rest of patients have the wild genotype (1/2→1/3). Compared with the wild-type group, the mutant group had a higher rate of diabetes mellitus, lower prevalence of drinking and alcohol consumption. The mutant group also showed a reduction in the coronary diameter and blood flow in response to sublingual administration of nitroglycerin, but the two groups were not statistically different in any other parameter. These results suggest that TRP12 and PHE15 are important for the function of apoA-IV in vivo.

**P118 Repair of Oxidative Low-density Lipoprotein and High-density Lipoprotein by Recombinant Human Methionine Sulfoxide Reductase A**

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**Background** Atherosclerosis(As) is characterized by the accumulation of both oxidized lipids and inflammatory cells in the arterial lesion that caused by a state of heightened oxidative stress. The existence of oxidized low density lipoprotein (LDL) and high density lipoprotein (HDL) in circulation could be used as potential markers for coronary artery disease. Methionine sulfone reductase A (MsrA) presents in all living organisms and is one of the most important antioxidative enzymes which reduce methionine sulfone (MetSO) residues in proteins. The goal of the study was to investigate the ability of recombinant human MsrA to repair oxidative damage of LDL and HDL in vitro, and protect against damage of cell induced by oxidized lipoproteins. **Methods and Results** The recombinant hMsrA was expressed in E. Coli (M15/SHpR4) and purified by Ni-NTA agarose affinity chromatography. Human LDL and HDL were separated by two-step gradient density ultracentrifugation and oxidized by Cu2+ and AAPH in vitro. Recombinant hMsrA incubated with lipoproteins could decrease the extent of electrophoresis mobility of ox-LDL and ox-HDL in agarose-gel electrophoresis, which denoted reduction of oxidative extent on LDL/HDL, hMsrA was also identified to protect apolipoprotein (apo) B100 of LDL against degradation by oxidation and restore the mobility of modification apo AI of HDL. Furthermore, hMsrA decreased the amount of MDA of LDL, indirectly reflecting its inhibitory ability on lipid peroxidation and protected cultured endothelial cells from injury induced by ox-HDL. **Conclusion** hMsrA may play an important role in protecting against lipoprotein oxidation and cell damage induced by ox-HDL, may account in part for the repairing function of hMsrA through reducing MetSO of apo AI and apo B100 to methionine.
Expression of Type IIA Secretory Phospholipase A2 Results in Severe Endothelial Dysfunction in Mice

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Endothelial dysfunction represents an early stage in the development of atheroscrotic cardiovascular disease, and is also considered as an independent predictor of cardiovascular disease mortality. The type IIA secretory phospholipase A2 (sPLA2) may actively contribute to atherogenesis, acting either locally within the arterial wall or in plasma. In the present study we tested the hypothesis that vessel wall expression of sPLA2 in transgenic mice results in endothelial dysfunction. We hypothesized that sPLA2 products contribute to pathogenesis of atherosclerosis by affecting the expression of multiple pro-inflammatory and pro-coagulant genes. To test this hypothesis, we incubated human aortic endothelial cell (HAEC) with either TGLR or TGLR lypolysis products for 3 hrs. TGLR were isolated from postprandial human plasma and incubated with lipoprotein lipase and the resulting lypolysis products were added to HAEC in culture. Total RNA was extracted from these cells and the TGLR or TGLR lypolysis products sensitive transcriptomes were obtained using high density oligonucleotide arrays (U133A 2.0 array). 266 of 285 genes were up-regulated and 19 genes were down-regulated by TGLR lypolysis products in comparison to TGLR alone. Functional classification of the affected genes identified transcription factors, cytokines, growth factors, cell cycle and inflammation-related genes. Specifically, TGLR lypolysis products up-regulated adhesion molecules (E-selectin and VCAM-1); cytokines (IL6, IL1α) and activating transcription factor 3 gene expressions. Expression of these genes was confirmed by quantitative real-time RT-PCR. Our results indicate that TGLR lypolysis initiate a stress response in HAEC that has the molecular signature of inflammation. These preliminary data from in vitro challenged of HAEC support our hypothesis showing that TGLR lypolysis products modulate multiple pro-inflammatory genes simultaneously that may contribute to the pathogenesis of atherosclerosis.

Antisense Reduction of ApoB-100 Favorably Modifies Lipoprotein Subclass Distribution in Healthy Human Volunteers

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Apolipoprotein B (apoB) is a key component of the atherogenic lipoprotein particles VLDL, IDL, and LDL. Elevated numbers of these atherogenic particles are associated with an increased risk for cardiovascular disease. An antisense inhibitor of human apoB, ISIS 301012, has demonstrated prolonged dose-dependent reduction in apoB in human clinical trials. Favorable effects on VLDL and LDL particle numbers were observed in a Phase 1, Double-blind, placebo-controlled, dose escalation trial involving 36 healthy volunteers with primary hypercholesterolemia. Subjects received an initial single dose of ISIS 301012 (50 to 400 mg) or placebo, followed by a 4-week multiple-dosing regimen at the same dose. Fasting lipoprotein particle numbers were determined using a large scale NMR spectroscopy platform (NMRall) on medium and small VLDL and HDL, and large and small LDL particle subclasses were assessed. Wilcoxon rank-sum tests were used to determine significant differences between placebo and the ISIS 301012-treated groups. A dose-dependent reduction in apoB-containing lipoprotein particle numbers was observed in subjects treated with ISIS 301012 where total, LDL and VLDL particle numbers were also significantly reduced by 63% (p = 0.02) and 66% (p = 0.004) in this group. Total LDL particle numbers were significantly reduced from baseline two weeks post-treatment in the 200 mg dose group (n = 8) by a median 36% (p = 0.04) and 54% (p = 0.0033) respectively. Small VLDL and LDL particle numbers were also significantly reduced by 61% (p = 0.05) and 69% (p = 0.004) in this group. Total LDL particle numbers were significantly reduced in the 100 mg dose group (n = 8). These results demonstrate a substantial reduction in atherogenic lipoprotein particle numbers by antisense inhibition of apoB. Due to the limited sample size and narrowly defined subject population, further studies will be required to validate these effects and determine whether the results differ depending on the nature of the dyslipidemia.

Statin Therapy Alters the Relationship Between ApoB and Lipoprotein Cholesterol and Non–High-Density Lipoprotein Cholesterol Targets in High-Risk Patients: MERCURY II Trial

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Background: Hypercholesterolemia patients may reduce their LDL-C to a predefined goal yet still have an excess of atherogenic lipoproteins. ApoB (apo B) provides a measure of both atherogenic lipoproteins and may be a superior predictor for CHD events. An apo B target of <90 mg/dL has been proposed as an alternative to non-HDL-C <130 mg/dL, particularly for hypertriglyceridemic (high TG) patients. This analysis examined what levels of LDL-C and non-HDL-C correspond to an apo B of 90 mg/dL.

Methods: The 16-wk MERCURY II trial examined patients with LDL-C <130 mg/dL, TG <400 mg/dL, at high risk for CHD (CHD, diabetes or 10-year Framingham risk >20%). LDL-C, non-HDL-C, TG, and apo B were analyzed at baseline and after statin therapy consisting of rosuvastatin (10 or 20 mg), atorvastatin (10 or 20 mg), or simvastatin (20 or 40 mg). For these new analyses, data from all patients were pooled to determine relationships between apo B and LDL-C and non-HDL-C, and linear regression analyses were done on values from baseline and after 16 wks of statin therapy.

Results: In high TG patients (TG ≥200 mg/dL, n = 656) and in low TG patients (n = 1128), baseline apo B was linearly related to baseline non-HDL-C and also linearly related to baseline LDL-C (Table). Target lipid values calculated from apo B and lipid data from untreated patients approximated the target values suggested for high-risk high TG patients by the NCEP in 2001. On statin therapy, apo B also correlated well with non-HDL-C and LDL-C (Table). However, target lipid values calculated from statin-treated patients were approximately 30 mg/dL lower than those from baseline data.

Linear Regression Slope Intercept (mg/dL) r Lipid value for apo B = 90 mg/dL

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Slope</th>
<th>Intercept (mg/dL)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo B vs non-HDL-C</td>
<td>Low TG baseline</td>
<td>1.02</td>
<td>38.8</td>
</tr>
<tr>
<td></td>
<td>High TG baseline</td>
<td>1.02</td>
<td>44.1</td>
</tr>
<tr>
<td></td>
<td>Low on statin</td>
<td>1.27</td>
<td>-10.0</td>
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<tr>
<td></td>
<td>High on statin</td>
<td>1.25</td>
<td>8.3</td>
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<tr>
<td>Apo B vs LDL-C</td>
<td>Low TG baseline</td>
<td>0.89</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>High TG baseline</td>
<td>0.92</td>
<td>110.4</td>
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<tr>
<td></td>
<td>Low on statin</td>
<td>1.11</td>
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</tr>
<tr>
<td></td>
<td>High on statin</td>
<td>1.03</td>
<td>-21.2</td>
</tr>
</tbody>
</table>

Conclusion: These data show that before therapy, an apo B <90 mg/dL approximated an LDL-C <100 mg/dL and a non-HDL-C <130 mg/dL. However, during statin therapy, an apo B target of <90 mg/dL in these high-risk patients corresponded more closely to the optional targets of LDL-C <70 mg/dL and non-HDL-C <100 mg/dL recommended for very high risk patients.

Novel Variants of Endothelial Lipase in Subjects with Elevated High-Density Lipoprotein Cholesterol

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Background: High levels of high density lipoprotein cholesterol (HDL-C) are associated with decreased risk of cardiovascular disease. Endothelial lipase (EL) is a recently discovered phospholipase in mice that influences HDL metabolism in vivo; overexpression causes reduced HDL-C and loss-of-expression causes increased HDL-C. We hypothesized that loss-of-function mutations in EL might cause high HDL-C levels in humans and therefore sequenced the EL gene in 96 subjects with HDL-C levels >99th percentile. A total of 32 gene variants were found, of which 17 were novel. One variant, confirmed by restriction analysis, was X501R, resulting in the conversion of the stop codon to an arginine and a predicted additional 49 amino acids at the carboxyterminus. There were 4 novel variants in the promoter (-662CG, -655CG, -576AG, -526CT) and 2 novel variants in the 3' UTR (C772T, G824A). Several previously undiscovered variants within the intronic regions were identified as well.

Conclusions: In subjects with high HDL-C levels, seventeen novel variants in the EL gene were identified, of which one is a novel missense mutation of the stop codon.
evidence of a dose-related effect. LDL-apoB production rate (PR) fell significantly with rosuvastatin 40mg/day, with no change in VLDL and IDL-apoB PR. Changes in TG were correlated with changes in VLDL apoB FCR and apoC-III, and changes in leithosterol:cholesterol ratio were correlated with changes in LDL apoB FCR, the associations being more significant with the higher dose of rosuvastatin. Rosuvastatin dose-dependent increase in HDL cholesterol (HDL-C) and Lp(a) concentration in a particle size profile. These effects were associated with a significant dose-related reduction Lp(a)-FCR with no changes to Lp(a)-PR. There was a significant dose-dependent reduction in Lp(a)-A1-FCR with concomitant reduction in Lp(a)-A1-PR, and hence no change in Lp(a)-A1 concentration. These data suggest that rosuvastatin decreases the plasma concentration of apoB-containing lipoprotein, via a mechanism that is dose-dependently related to an increase in their rates of catabolism. Furthermore, rosuvastatin increases HDL cholesterol and Lp(a) concentrations and this was primarily related to reduction in Lp(a) fractional catabolic rate. The findings provide a dose-related mechanism for the benefits of rosuvastatin on cardiovascular disease in the metabolic syndrome.

Granzyme B: A Major Contributor to Atherosclerosis, Xanthomatosis, and Calcification in the Egfr Waved-2 Mouse Model of Aortic Stenosis

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Left ventricular hypertrophy (LVH) is an independent risk factor for cardiovascular morbidity and mortality. LVH associated with aortic stenosis (AS) is an initial cardiac response to pressure overload. However, variation exists in the geometric distribution of LVH and cardiac compensation in patients with similar AS. Moreover, up to 10% of patients with severe AS never develop LVH. Several clinical risk factors influencing LVH in AS, yet few genetic markers have been identified. Mice homzygous for the homzygous Egfr waved-2 (wa2) allele were reported to have LVH on a mixed genetic background. We created C57BL/6J (B6), 129S1 (129), and B6.129 (F1) Egfr+/− mice to investigate the effect of genetic factors on the cardiac response to chronic AS. At three months of age, all Egfr+/− mice have enlarged aortic valves relative to Egfr−/− littermates. However, B6 Egfr+/− mice have the thickest valves, with significant transvalvular gradients as assessed by cardiac catheterization (38±8 mmHg), enlarged, fibrotic hearts, hypertrophied cardiomyocytes and a reduced lifespan. These mice also have severely reduced systolic function (%FS: F1 Egfr+/− 40.3±11.2 vs. 41.2±7.3 Egfr−/−) and altered expression of classic cardiac hypertrophy markers. Despite having enlarged valves, neither 129S1 nor F1-Egfr+/− mice develop histological, functional or molecular evidence of LVH and maintain normal systolic function (%FS; F1 Egfr−/− 40.3±11.2 vs. 129 Egfr−/− 41.3±6.5). Histological studies reveal significant cellular changes in cardiac valves unique to B6 Egfr+/− mice (increased cellular proliferation, ectopic cartilage formation, extensive calcification and inflammatory infiltrate) which may contribute to altered valve physiology. These results confirm the usefulness of this model to study AS and LVH. Additionally, identification of 129 genetic modifiers may uncover novel therapeutic targets for the treatment of calcific AS.

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Reduction of Plateau Size and 5LO, ALOX5P, and LTA4H Gene Expression in a Dyslipidemic Mouse Model of Atherosclerosis by DG-031, an Inhibitor of Leukotriene Biosynthesis

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Background. Previously we reported that DNA variants in the ALOX5P and LTA4H genes encoding 5-lipoxygenase activating protein (FLAP) and leukotriene A4 hydrolase (LTA4H), respectively, key enzymes in the leukotriene biosynthetic pathway, are associated with increased risk of myocardial infarction and stroke (Helgadottir et al., 2005 & 2006). Other groups independently report that expression of 5-lipoxygenase (5LO), FLAP and LTA4H is elevated in human atherosclerotic plaque and also in the aorta of dyslipidemic mice. Here we evaluate the effect of pharmacological inhibition of FLAP in a dyslipidemic mouse model of atherosclerosis. Methods. ApoE−/− mice were administered DG-031, an inhibitor of FLAP, in a high fat diet from 8–16 weeks of age. Aortas were harvested for measurement of mRNA content by TaqMan analysis or atherosclerotic plaque burden in aortic roots. Results. Oil red O stained cross sections from the aortic root we demonstrate that DG-031 causes a dose dependent reduction in atherosclerosis of about 30% (p<0.01). Accompanying the reduction in atherosclerotic plaque formation was a significant reduction in aortic 5LO, ALOX5P, and LTA4H gene expression of greater than 50% (p<0.01). DG-031 also reduced the increase in expression of atherosclerotic lesion type-3 and 9 (MMP3 and MMP9) genes that are found in atherosclerotic lesions (p<0.05).

Conclusions. DG-031, a FLAP inhibitor, currently being studied in a Phill clinical trial for secondary prevention of CV events in post-ACS patients, provides dose-dependent reduction in atherosclerotic lesion formation and significant decrease in aortic expression of leukotriene pathway genes in dyslipidemic apoE−/− mice.

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The Effect of Sphingomyelin Synthase Gene Knockdown and Knockout on Plasma Membrane Sphingomyelin Levels and NF-κB Activation

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Sphingomyelin (SM) is one of the major lipid components in plasma and on cell membranes. Two isoforms of SMs (SM1 and SM2) are involved in the biosynthesis of SM. To investigate the relationship between SMs and NFκB, we used gene knockout and knockdown approaches. We found that SM1 and SM2 siRNA significantly decreased intracellular [3H]-SM levels by about 55% and 27%, respectively. To investigate the consequence of SM inhibition, we incubated the siRNA-transfected Hu7 cells with [3H]-L-serine, a precursor of sphingomyelin and found that both SM1 and SM2 siRNA significantly decreased intracellular [3H]-SM levels compared with controls. LC/MS indicated that both SM1 and SM2 knocked down significantly reduced cellular SM but not ceramide, S1P and sphingosine levels. In order to study the impact of SM knockdown on lipid rafts, we isolated lipid rafts and determine SM and cholesterol levels in them. We found that SM1 and SM2 knockdown significantly decreased SM but not cholesterol levels in lipid raft fractions. This effect was not observed in non-raft lipid fractions. Furthermore, SMs siRNA-treated cells exhibited stronger resistance to cell cytes lysed by lysisen, an SM aggregate binding protein, than did control siRNA-treated cells. More importantly, complete Sm2 gene knockout (KO), significantly reduced lysisen-mediated macrophage lysis. These results suggest that SM plays a significant role in regulating SM-containing membrane microdomains which play important roles in cell signaling. Indeed, immunocytochemistry, luciferase and western blot analysis indicated that the activation of NF-kappaB was attenuated in SMs knocked down cells treated with TGF-β, and in SMs KO macrophages treated with LPS. Moreover, this was less evident in the mRNA and protein levels of the inflammatory genes, TNF-α, IL-6, and IL-1β, in LPS treated SMs KO macrophages that were from wild type mice. Taken together, our data suggest that SM1 and SM2 not only regulate intracellular and plasma membrane SM levels but also play important role in cell signaling pathways, such as inflammation, which may well have an impact on the development of atherosclerosis.

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Cellular Mechanisms Regulating Cholesterol Ester Transfer Protein-mediated Selective Uptake of High Density Lipoprotein–Derived Cholesterol Esters

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Cholesterol ester transfer protein (CETP) is a hydrophobic glycoprotein that mediates the transfer of neutral lipids between lipoproteins. Recently, our laboratory has demonstrated a novel role for CETP in directly mediating the selective acquisition of CE from HDL by hepatocytes, indicating a direct and potentially anti-atherogenic function in reverse cholesterol transport. Further studies have indicated that CETP-mediated uptake is not dependent on CETP-mediated selective uptake of HDL-CE. Using biochemical plasma membrane isolation followed by detergent extraction, we demonstrated that CETP localizes in the low density, detergent-resistant membrane fractions in both ODS-7 cells and primary murine hepatocytes infected with an adenoviral CETP construct. In an attempt to dissect the intracellular events following the selective uptake of HDL derived CE mediated by CETP, immunofluorescence confocal microscopy was used. By incubating Hepa cells and primary hepatocytes expressing CETP with fluorescein labeled HDL and CE, we demonstrate that CETP colocalizes with both CE and HDL on the cell surface and in a subcellular population consistent with the G2D-HDL complex. We also note that the CETP/HDL complex colocalizes with a subset of early endosomes, suggesting a possible raft-mediated endocytic route, consistent with CETP’s non-caveolar raft localization. At 1h post treatment, HDL and CETP were seen to separate from the early endosomes and segregate in structures that have yet to be identified. We speculate that HDL and CETP may be recycled through a retromembranous pathway, during which CE may...
be removed from the HDL particle and directed to the lipid droplets for storage. These studies provide new insight into CETP membrane localization and intracellular trafficking, relevant to its role in CE selective uptake.

WITHDRAWN

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Nuclear Bile Acid Receptor Gene Variant Improves Efficacy of Lipid-lowering Therapy

Atsushi Nohara, Hayato Tada, Shoji Katsumi, Masa-aki Kawashiri, Akihiru Inazu, Junji Kobayashi, Masakazu Yamagishi, Hiroshi Maubuchi; Kanazawa Univ Graduate Sch of Med Science, Kanazawa, Japan

Bile acid receptor FXR has crucial roles in cholesterol conversion into bile acids and in its recycling through many target genes that may affect cholesterol levels. We have identified common polymorphism -1g→-1a in FXR gene in Japanese population, and hypothesized that this polymorphism could affect lipid-lowering therapy response. Methods and Results: Total 278 patients (M/F=147/129) suspected CAD were enrolled. FXR -1g→-1a genotypes were determined by PCR-RFLP, and -1t allele frequency was 0.32. Lipid-lowering drugs (statins 92%) were prescribed in 113 patients (M/F=66/67), and baseline Tcho level was not different with FXR genotype (p=0.65 vs. gt+tt). Lipid analysis, lipids level was not different between groups (p=0.11, p=0.15, p=0.008). We also found that FXR polymorphism affects the activity of key enzymes involved in cholesterol metabolism such as CYP7A1. Whether this variant could affect long-term clinical course should further be sought.

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Epoxysterol Cholesterol Treatment Increases ABCA1-Mediated Cholesterol Efflux in LDL- but Not Acetylated LDL-loaded Macrophages

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BACKGROUND: We recently showed that LDL cholesterol preferentially effluxed to HDL, whereas a modified acetylated LDL (AcLDL), primarily effluxed to lipid-poor apoA-I in an ABCA1-dependent fashion in murine bone marrow derived macrophages. While the intracellular cholesterol trafficking pathways are clearly different, the regulatory mechanisms remain unclear. Here we studied how the LXR activation regulated cholesterol transport through these two pathways. METHODS AND RESULTS: LDL and AcLDL labeled macrophages were treated with the LXR ligand, 9-cis Retinoic acid (9-cis RA), and the activity, protein expression and mRNA for ACAT2 were all decreased in response to 9-cis RA treatment. The degree of cholesterol synthesis inhibition, marked induction of LDL receptors and HMG CoA reductase was detected and positively related to the degree of cholesterol synthesis inhibition. In a corresponding way, we could show that the activity, protein expression and mRNA for ACAT2 were all decreased in response to cholesterol synthesis inhibition. The lowering of HDL cholesterol was shown in response to high-dose statin treatment could not be explained by changes in structures such as the HDL receptor CLA-1, ABCA1 or apoA-I. We conclude that ACT2 activity in human liver is lowered by cholesterol synthesis inhibition, and that this effect, in combination with a parallel down regulation of Apo E expression, may contribute to the favourable lowering of VLDL cholesterol seen in addition to the LDL lowering during statin treatment.

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Glycoprotein Ib/IIa and Coronary Disease Extension

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Glycoprotein Ib-llla plays a pivotal role in the connection to the platelets of fibrinogen and other adhesion proteins. The gene that codifies sub unit III a, with two alleles PLAT and PLAA, has already been identified. The PLAA allele has been associated to acute coronary syndrome (ACS) emergence, and individuals possessing the PLAA allele would bind more fibrinogen to platelets than do PL A1 homozygotes. The response to thrombin can differ between the two genotypes. PLAA allele individuals could have less severe, less complex and less extensive coronary morphology lesions and more reactive platelets. Aims: to evaluate the influence of polymorphism of glycoprotein (GP) IIa gene (PLAT and PLAA) in coronary disease extension, in a Portuguese population. Methodology: In 276 consecutive coronary patients who underwent coronary angiography for diagnostic purposes, was evaluated the Leaman coronary score in the GP IIa polytype PLAT-PLA1: PLAT-PLA2, and PLAT-PLA2. The average genotype scores were compared by Student T test for independent samples (unilateral analysis, p<0.05). Results: In this coronary Portuguese population the GP IIa genotype PLAT/PLA1 PL A2 presented less severe coronary artery morphology lesions, which was also statistically significant. Looking at these results we can conceive that there are coronary patients with severely altered coronary morphology, in whom the antiplatelet factor is predominant over others with relatively preserved coronary morphology in whom the pro-aggregative and thrombotic factors dominate. In patients with platelet aggregation alterations, anti-aggregation therapy will be particularly important.

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Reduced Hepatic AGAT2 Activity Is Part of the Integrated Response to Inhibition of Cholesterol Synthesis in Humans

Paolo Parini, Ulf Gustafsson, Karolinska Institute, Stockholm, Sweden; Matt Davis, Wake Forest Univ, Winston-Salem, NC; Staffan Sahlin, Bo Angelin, Karolinska Institute, Stockholm, Sweden; Larry L Rudel, Wake Forest Univ, Stockholm, NC; Mats Eriksson, Karolinska Institute, Stockholm, Sweden

In order to identify how different degrees of cholesterol synthesis inhibition affects human hepatic cholesterol metabolism, we studied 37 normo-cholesterolemic gallstone patients randomized to treatment with placebo, 20 mg/dl fluvastatin or 80 mg/dl atorvastatin for 4 weeks prior to surgery. Based on serum lathosterol determinations, cholesterol synthesis was reduced by 42% and 70% in the two groups receiving statins. During gallstone operation a liver biopsy was taken, and hepatic protein and mRNA expression of several rate-limiting steps of cholesterol metabolism were assayed and related to the induced serum lipoprotein changes. A marked induction of LDL receptors and HMG CoA reductase was detected and positively related to the degree of cholesterol synthesis inhibition. In a corresponding way, we could show that the activity, protein expression and mRNA for ACAT2 were all decreased in response to cholesterol synthesis inhibition. The lowering of HDL cholesterol was shown in response to high-dose statin treatment could not be explained by changes in structures such as the HDL receptor CLA-1, ABCA1 or apoA-I. We conclude that ACT2 activity in human liver is lowered by cholesterol synthesis inhibition, and that this effect , in combination with a parallel down regulation of Apo E expression, may contribute to the favourable lowering of VLDL cholesterol seen in addition to the LDL lowering during statin treatment.

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Aging Increases the Inflammatory Response After Vascular Injury, Leading to an Exaggerated Neointimal Formation

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Aging is a risk factor for the development of vascular occlusive diseases. The aim of this study is to determine whether aging prolong the inflammatory response after vascular injury , leading to an exaggerated neointimal development. Using iliac balloon injury model, we found that arteries from old rats (22 month) developed thicker neointima at 4 weeks after injury than those from young (4 month) ones (IM 8.8 ± 0.2 vs 5.4 ± 0.15, p<0.05). We also found that old arteries accumulated more alpha-actin + vascular smooth muscle cells in the neointima than their younger counterparts (500 ± 100 vs 320 ± 120 cell/mm²). Although there is similar number of macrophages (CD68+ cells, detected by immunohistochemistry, IHC), in the adventitia 4h after injury in young and old aging rats , these cells disappear in the former after 3 days, while remain in the media and neointima of the latter up until 30 days after injury. Interestingly, the macrophages in old arteries are CD163 negative, indicating they are of pro-inflammatory phenotype. There are no difference in the number of vascular T cells and dendritic cells between young and old rats after injury. The vascular cytokines production was assessed at different time points after balloon injury using the LincoPlex system. Old arteries contained three folds or more IL18, IL-6, Gro KC, and Leptin than the young ones as early as 3 days after injury. These pro-inflammatory cytokines stayed elevated in the old vasculature for most of the arterial remodeling process, in contrast with the old arteries, young arteries produced three times or higher levels of anti-inflammatory cytokines -IL-10, IL-17, IL13 and
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Number of Nitrate Groups Determines Reactivity and Potency of Organic Nitrates: A Proof of Concept Study in ALDH-2/- Mice

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Background and purpose: Mitochondrial aldehyde dehydrogenase (ALDH-2) has been shown to provide a pathway for bioactivation of organic nitrates and to be prone to desensitization in response to highly potent, but not to less potent nitrates. We therefore sought to strengthen the concept, that bioactivation by ALDH-2 critically depends on the amount of nitrate groups within the nitrovasodilator. Experimental approach: Nitrates with one (PEMN), two (PEDN, GDN), three (PETN; glyceryl trinitrate, GTN) and four (pentamethylenetetranitramine, PMTN) nitrate groups were investigated. Vasodilatory potency was measured in mesenteric vasoconstriction studies using isolated aortic segments of wild type (WT) and ALDH-2/- mice. Activity of the cGMP-dependent kinase-I (reflected by levels of phosphorylated Vasodilator Stimulated Phosphoprotein, P-VASP) was quantified by Western Blot analysis, mitochondrial dehydrogenase activity by HPLC. Following incubation of isolated mitochondria with PETN, PETN-Hydrate and GTN, C0 and N0 metabolites were quantified using chemiluminescence nitrogen detection and mass spectrometry. Key results: Compared to WT, vasorelaxation in response to PETN, PETN-Hydn and GTN was attenuated about 10fold in ALDH-2/- mice, identical to WT vessels preincubated with inhibitors of ALDH-2. Reduced vasodilatory effect correlated with reduced P-VASP formation and diminished biotransformation of the tetranitrate- and trinitrate-compounds. Intriguingly, none of these findings were observed for PEDN, GDN and PEMN. Conclusions and implications: Our results support the crucial role of ALDH-2 in bioactivating highly reactive nitrates like PETN and PETN-Hydn. ALDH-2 appears to act as a gatekeeper for the number of nitrate groups. Less potent nitrates like PEDN, GDN and PEMN are apparently biotransformed by alternative pathways.

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Positive Association of C667T Methylenetetrahydrofolate Reductase Gene Polymorphism with Acute Ischemic Stroke

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BACKGROUND AND PURPOSE: Elevated plasma homocysteine (Hcy) is a risk factor for ischemic stroke and coronary heart diseases. Polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene are associated with high plasma Hcy levels in various populations. However, no large-scale studies have been reported to have a protective effect in endothelial cells. Nicotinamide 3 oxide 3 (NOS3) is a candidate gene for stroke susceptibility. We investigated the association of C667T mutation of MTHFR gene and three NOS3 polymorphisms (G974T missense variant in exon 7, variable number tandem repeats in intron 4, and T786C in the promoter) with acute ischemic stroke in a Singapore population. METHODS: Patients with acute ischemic stroke (n=120, 60.8% ethnic Chinese) were studied. Two hundred and seven subjects (74.4% ethnic Chinese) with no stroke history of stroke served as controls. Hypertension, diabetes, dyslipidemia and age were significant risk factors associated with acute ischemic stroke. Analysis of stroke patients as compared to controls on the number of nitrate groups. Less potent nitrates like PEDN, GDN and PEMN are apparently biotransformed by alternative pathways.

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Piglitazone Increases Macrophage Apoptosis and Plaque Necrosis in Advanced Atherosclerotic Lesions of LDL Receptor–Deficient Mice

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Thiazolidinediones (TZDs), whose actions involve both PPAR dependent and independent effects, improve insulin sensitivity in type II diabetes and inhibit early foam-cell atherogenesis in mice. However, the effects of TZDs on advanced lesion progression are unknown. Here, we explore the effects of TZDs in vitro on two processes critical in plaque necrosis, namely, macrophage apoptosis and phagocytic clearance (efficacyosis) of apoptotic macrophages, and in vivo on advanced atherosclerotic plaque progression. In cell culture studies, the TZDs pioglitazone and rosiglitazone enhanced FC-induced macrophage apoptosis as well as efficacysis of apoptotic macrophages by macrophage phagocytes. Because enhancement of advanced lesion macrophage apoptosis would be expected to promote plaque necrosis, whereas enhanced efficacyosis would be predicted to be beneficial, the net effect in vivo was explored. For this purpose, non-diabetic LDL–/- mice were first fed a cholesterol-enriched diet to promote mid-stage lesions, and then pioglitazone was administered along with diet for an additional 10 weeks. Despite a decrease in plasma non-HDL cholesterol and an increase in HDL cholesterol, plaques from pioglitazone-treated mice were more necrotic and displayed increased apoptotic cells in macrophage-rich regions. Thus, the beneficial effect of commonly used TZDs on insulin resistance may be partially offset by adverse effects of advanced plaque progression.

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Cannabinoid Receptor (CB2) Deficiency Inhibits Oxidized LDL/Oxysterol-induced Apoptosis in Macrophages

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Apoptosis of macrophages is an important event in the pathophysiology of atherosclerosis. Oxidized low-density lipoproteins (OxLDL) are a major lipid component of atherosclerotic lesions and endocytosis of OxLDL is a potent inducer of apoptosis in cultured macrophages. OxLDL-induced apoptosis in macrophages is largely due to the oxidized cholesterol derivatives, such as 7-ketocolesterol (7kC), in the OxLDL. Cannabinoids exert their effects through two related G-protein coupled receptors, CB1 and CB2. CB2 is primarily expressed by cells of the immune system and several macrophage processes associated with ongoing atherogenesis including proliferation, secretion of inflammatory cytokines and chemokines, are regulated by CB2. Expression of CB2 has been detected in lesions and a low dose of the cannabinoid, l-3-tetrahydrocannabinol, reduces plaque size in ApoE–/- mice. Herein, we have tested the hypothesis that CB2 deficiency increases OxLDL-induced macrophage apoptosis. To test this possibility aortic macrophages were isolated from CB2(+/+) and CB2(–/-) mice. The macrophages were cultured with 7kC for 16h with 50 μg/ml OxLDL, in situ terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) analysis detected significantly less CB2(–/-) macrophages undergoing apoptosis than CB2(+/+) controls (27.9 ± 4.7% vs 61.9 ± 8.5%, p < 0.001). Treatment of CB2(–/-) macrophages with 7kC also resulted in significantly fewer apoptotic cells compared to similarly treated wild type macrophages (18.9 ± 10.5% vs 54.1 ± 6.9%, p < 0.001). Furthermore, reduced cleavage of caspase-3 and PARP in CB2(–/-) macrophages treated with 7kC compared to CB2(+/+) controls, a result which was confirmed by caspase-3 activity assays. In contrast, staurosporine-induced apoptosis was unaffected by CB2 deficiency consistent with the conclusion that CB2(–/-) macrophages are not generally defective in apoptosis. From these results we conclude that CB2 deficiency is associated with reduced OxLDL-induced apoptosis and suggest that one mechanism by which CB2 influences the development and progression of atherosclerotic lesions is by mediating the apoptotic response of macrophages to OxLDL.

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New Insights into the Roles of Apolipoprotein C-III in Stimulating the Production of Hepatic VLDL

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Apolipoprotein C-III is a constituent of VLDL and plays a role in regulating lipoprotein lipase activity as well as receptor-mediated lipoprotein endocytosis. We reported previously that stable expression of apoC-III in MCA-R7777 cells promoted VLDL-triaclyglycerol (TG) secretion, but the effect of apoC-III expression on the synthesis or secretion of apoB100, the structural protein of VLDL, was not examined. In this study we determined the rate of apoB100 translation and secretion using MCA-R7777 cells transiently transfected with apoC-III. As observed in stables, transient apoC-III expression increased by (2-fold) secretion of [35S]glycerol-labeled TG and phosphatidycholine (PC) associated with TG-rich VLDL (Sf > 100). In addition, transient apoC-III expression also resulted in increased incorporation of [35S]methionine into cell-associated and secreted VLDL/LDL-apoB100 by 2-fold, respectively. Moreover, transient apoC-III expression increased post-translational stability of 35S-apoB100; thus pulse-chase analysis showed that the recovery of total 35S-apoB100 cell (cell + media) was increased by 25% in apoC-III expressing cells. Intracellular degradation of 35S-apoB100 could be blocked by MG132 in control cells, but was less sensitive to MG132 in apoC-III expressing cells, indicating that apoC-III expression diminished proteosome-mediated apoB100 degradation. The increased apoB100 synthesis/ssecretion suggested greater lipid availability for VLDL assembly. In apoB100 expressing cells, subcellular fractionation showed that partitioning of 3H-TG into microsomal lipid was markedly augmented, and 3H-TG accumulation in cytosolic lipid droplets was decreased in apoC-III cells. The ability of apoC-III to promote 3H-TG partitioning was tested by expressing an apoC-III variant (designated apoC-III PD) in which the apoC-III PD was secreted normally. Unexpectedly, not only did expression of apoC-III PD stimulate 3H-TG secretion (by 1.5-fold) but also the expressed apoC-III PD was secreted normally. Thus, apoC-III possessed a unique effect of apoC-III on VLDL assembly and secretion.

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Divergence in Vascular Fractalkine Expression and Functional Responses in Male and Female Spontaneously Hypertensive Rats

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Men have a greater incidence of cardiovascular disease compared to women, however the molecular mechanisms responsible for cardiovascular protection in females are unknown.
Cholesterol efflux is essential for macrophage cholesterol homeostasis through reverse cholesterol transport via extracellular acceptors such as apolipoprotein A-I (apo-A-I) and high density lipoprotein (HDL). We have previously reported that EP 30317 induces a significant decrease in macrophage foam cell formation. Hypothesis. Our hypothesis is that EP 30317 regulates macrophage cholesterol efflux through the PPAR-γ-LXRb-ABC pathway. In the present study, the role of nuclear receptors and of the different ABC transporters in EP 30317-mediated cholesterol efflux has been evaluated. Methods. Murine monocytic J774 cells were loaded with [3H]-cholesterol (1 μCi/ml), incubated with EP 30317 (100 μM) and exposed to HDL (apo-A-I or apo-A-I) and SR-A or SR-B1 agonists in order to promote efflux. Results. With SR-A agonist, EP 30317 induced a significant increase of cholesterol efflux by 163% (p<0.001) after 4 and 16 hours, respectively. In contrast, EP 30317-mediated efflux to HDL increased only by 32–26% (p<0.001), under the same conditions. The significant increase of EP 30317-mediated cholesterol efflux was found. The expression of proteins involved in cholesterol efflux, as assessed by Western blot, was increased by 2.5-, 2.2- and 7.3-fold for LRxRα, ABCG1 and ABCA1, respectively, without significant change in the PPAR-γ protein level. Conclusion. EP 30317 elicits PPAR-γ-dependent cholesterol efflux from J774 cells to HDL and apo-A-I as cholesterol acceptors in the reverse cholesterol transport.

A Specific Role for Cytoplasmic Amino Acid Sequences in Regulating SR-A Function

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Macrophage class A scavenger receptors (SR-A) are transmembrane receptors that recognize diverse ligands including modified lipoproteins, bacterial products, and modified extracellular matrix (ECM). SR-A internalizes soluble ligands and enhances cell adhesion to modified ECM. The cytoplasmic domains required for mediating SR-A internalization and cell adhesion have not been determined. In this study, we mutated the cytoplasmic tail of SR-A and stably expressed the altered receptors in a human embryonic kidney (HEK 293) cell system. Deletion of all but six cytoplasmic amino acids (SR-AΔ6) eliminated receptor internalization while increasing surface localization and cell adhesion. To examine the importance of specific sequences in the cytoplasmic tail, we created an SR-A mutant with amide derivatization of 4 highly conserved cytoplasmic acidic amino acids (SR-AΔ6-P143–146). Similar to SR-AΔ6, SR-AΔ6-P143 displayed increased surface localization and cell adhesion however SR-AΔ6-P143 showed no internalization of the novel ligand. To create an SR-A receptor that only internalizes ligand, we created a chimeric receptor in which the cytoplasmic tail of SR-A was replaced by the first 57 amino acids, including the internalization motif of the transferrin receptor (SR-AΔ6-TfR). In contrast to SR-AΔ6-TfR, cells expressing the SR-AΔ6-TfR internalized ligand but did not show enhanced cell adhesion. These results indicate a strong correlation between surface localization and cell adhesion. Because P3-Kinase (PI3-K) is involved in receptor trafficking we examined the importance of PI3-K in SR-A-mediated adhesion. Although PI3-K is activated during SR-A-mediated adhesion, PI3-K activity is required only for SR-A-mediated adhesion in cells expressing full-length SR-A. Together, these results indicate that SR-A function can be modulated by the PI3-K-dependent regulation of receptor localization.

LXR Activation by 24(S,25)-Epoxycycholesterol Enhances the Expression of Niemann-Pick C1 in Macrophages, Leading to Increased Cholesterol Efflux

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Niemann-Pick C (NPC) and NPC2 are implicated in the trafficking of cholesterol between lysosomal compartments, the endoplasmic reticulum and the plasma membrane for efflux to extracellular acceptors. Deficiency of Niemann-Pick C1 (NPC1) and NPC2 are responsible for Niemann-Pick disease types A and B, respectively. The synthetic LXR agonist, TO-901317 was shown to enhance expression of both NPC1 and NPC2 in a PPARγ-dependent manner, contributing to enhanced cholesterol efflux.

In silico analysis of NPC1 and NPC2 revealed a DR4 motif for each gene potentially capable of binding LXR and modulating gene expression. Exposure of THP-1 cells to an OSCi ligand and bone marrow transduction. There was no significant difference in total cholesterol reduction in macrophages.

Together, these results indicate that SR-A function can be modulated by the PI3-K-dependent regulation of receptor localization.
lysozymes hampers v-ATPase and inhibits the ability of lysozymes to maintain an active pH. This could exacerbate foam cell formation and influence atheromatous lesion development.

The Association Between Alcohol Consumption and C-Reactive Protein Levels in College-aged Individuals

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Purpose. Current screening methods fail to identify over half of individuals at risk for cardiovascular disease. C-Reactive protein (CRP), an acute phase protein and marker for inflammation, is highly correlated with cardiovascular disease and is a promising new screening tool. Many factors, such as alcohol, medications, physical activity, and excess body fat, affect CRP levels. Alcohol intake results in a J-shaped response curve for CRP in individuals over forty years. This study examines the effect of alcohol consumption on CRP levels in college-aged individuals. Binge drinking is prevalent in college-aged individuals potentially increasing CRP levels. Design. College-aged individuals completed surveys which assessed factors that may affect CRP levels, such as medication use, smoking habits, recreational alcohol and alcohol consumption patterns. Three groups, non-drinkers (N=6), moderate drinkers (N=10), and heavy drinkers (N=9), were matched based on survey responses. C-Reactive protein (hs-CRP) was measured using reflectance photometry. This research was approved by the university’s institutional review board. The average level of all alcohol consumption patterns was 0.9 mg/L (low risk for cardiovascular disease). Moderate drinkers had significantly lower CRP levels (0.58mg/L) than heavy drinkers (1.25 mg/L; p = 0.041), but there was no significant difference between CRP levels of moderate drinkers and non-drinkers (0.85 mg/L; p = 0.343). Males had higher CRP levels than females, but this difference did not reach statistical significance (p = 0.09). Conclusions. There is a J-shaped relationship between alcohol consumption and CRP levels in college-aged individuals, agreeing with trends found in older adults. If CRP levels are predictive of future risk for cardiovascular disease, college aged individuals may be beginning this pattern, which is an additional reason to be concerned about heavy drinking in college-aged individuals.

Comparison of the Cellular Lipid Efflux Properties of Pre-β-High Density Lipoprotein and Lipid-poor Apolipoprotein A-I

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The ATP-binding cassette transporter A1 (ABCA1) mediates the efflux of cellular phospholipids (PL) and free (unesterified) cholesterol (FC) to human apo A-I to form nascent HDL particles. With J774 macrophages and human skin fibroblasts, the nascent HDL particles present after 24h incubations are discoidal, contain 2, 3 or 4 molecules of apo A-I per particle, possess α-helical, proline rich mobility and are about 9 and 12nm in diameter. These particles have PL/FC/apo A-I molar ratios ranging from 96–195/12–39/1. Here, we show that human skin fibroblasts and WI38VA13 (human lung fibroblasts), which release PL and FC to apo A-I relatively slowly, form precursor particles containing predominantly monomeric apo A-I. These small particles can be obtained by incubating lipid-poor apo A-I (15µg/ml) for 1–2h with either human skin fibroblasts or WI38VA13 cells in which ABCA1 is up-regulated. The hydrodynamic diameter of these particles is ~7 nm and they exhibit pre-β electrophoretic mobility as assessed by non-denaturing 2D-PAGE. With both cell types, the PL/apoA-I molar ratio for these particles, which contain one apo A-I molecule (as assessed by covalent cross-linking), is in the range of 3–4/1 and the FC/apoA-I molar ratio is 1–2. SDS-PAGE of concentrated samples did not reveal any evidence of other proteins besides apo A-I in these particles. These pre-β particles are the “lipid-poor” apo A-I frequently mentioned in the literature. When incubated with human skin fibroblasts in which ABCA1 is up-regulated, they effectively efflux FC with K1 Δ μg apo A-I/ml and Vmax = 6–7 ng cellular FC/ml. The equivalent values for lipid-poor apo A-I are very similar indicating that the pre-β HDL and lipid-poor apo A-I are equally effective in mediating FC efflux. In both cases, the apo A-I is converted to the 9 and 12nm discoidal particles upon further incubation with the cells. The above results suggest that discoidal HDL particles can be formed similarly by ABCA1-mediated addition of cellular lipids to either monomeric lipid-poor apo A-I molecules or monomeric lipid-poor apo A-I (pre-β particles). Knowledge of the reaction products will aid in the elucidation of the mechanism of ABCA1 action.

The Effect of Selective Inactivation of Macrophage ATP Binding Cassette Transporter A1 (ABCA1) on Atherosclerosis in LDL-receptor Knockout (LDLrKO) Mice

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Macrophage ABCA1 is thought to play a critical role in removal of excess cholesterol in atherosclerotic lesions. Previous studies have shown that transplantation of bone marrow from ABCA1 KO mice into LDLrKO recipient mice results in more atherosclerosis compared to transplantation of wild type bone marrow. However, bone marrow transplantation eliminates ABCA1 in all leukocytes, not just macrophages. We developed LDLrKO mice with targeted deletion of the apolipoprotein B promoter region (MSKO-LDLrKO) of macrophage ABCA1 in atherosclerosis development. Mice were fed an atherogenic diet (10% palm oil, 0.2% cholesterol) for 16 wk before aortic atherosclerosis was quantified as percentage of surface occupied by lesions as well as cholesterol content. Surprisingly, neither measure of atherosclerosis was different between the two genotypes of mice. Analysis of plasma cholesterol showed a significant reduction in apolipoprotein B lipoprotein concentration in plasma of MSKO-LDLrKO mice compared with LDLrKO mice, despite a significant two-fold increase in hepatic cholesterol in the MSKO-LDLrKO mice. Thiglycolate-elicited peritoneal macrophages from MSKO-LDLrKO mice on the atherogenic diet responded to lipopolysaccharide stimulation with greater proinflammatory cytokine (i.e., TNF-alpha, IL-6, IL-1beta) gene expression relative to macrophages from LDLrKO mice. These results suggest that deletion of macrophage ABCA1 in the LDLrKO background has two opposing outcomes with regard to atherosclerosis development, an increased macrophage proinflammatory response and a novel indirect role in increasing plasma VLDL and LDL catabolism and/or decreasing hepatic VLDL secretion. These opposing effects of macrophage ABCA1 may explain the similar atherosclerosis between LDLrKO mice with or without functional macrophage ABCA1.

SLX-4090, an Enterocyte-specific Microsomal Triglyceride Transport Protein Inhibitor, Lowers LDL Cholesterol and Triglycerides While Raising HDL Cholesterol in Apo E -/- Mice Fed a High-Fat Diet

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MTP facilitates the formation and transfer of chyomicrons and VLDL in the intestine and the liver respectively. Inhibiting both intestinal and hepatic MTP lowers serum levels of total, VLDL and LDL cholesterol as well as triglycerides but leads to hepatic lipodosis. SLX-4090 was designed to be enterocyte-specific and non-absorbable thus avoiding mechanism-based liver toxicity. Male Apo E -/- mice were fed a high fat diet for 10 weeks which contained either no SLX-4090 (control) or 0.006% (10 mg/kg/day) or 0.019% (30 mg/kg/day) or 0.06% (100 mg/kg/day) SLX-4090. SLX-4090 dose-dependently decreased total cholesterol and LDL levels while raising HDL levels.

<table>
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<tr>
<th>Treatment</th>
<th>Control</th>
<th>10 mg/kg SLX-4090</th>
<th>30 mg/kg SLX-4090</th>
<th>100 mg/kg SLX-4090</th>
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<tr>
<td>Total Cholesterol</td>
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<td>166±115</td>
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<td>523±54***</td>
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<tr>
<td>(mg/dL)</td>
<td></td>
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<tr>
<td>LDL (mg/dL)</td>
<td>157±45</td>
<td>161±112</td>
<td>908±131***</td>
<td>474±52***</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>16±1</td>
<td>22±1***</td>
<td>26±2***</td>
<td>35±2***</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
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<td>178±24</td>
<td>105±13***</td>
<td>70±8***</td>
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<td>Body Weight Gain (g)</td>
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<td>11.9±2.0</td>
<td>9.5±1.4**</td>
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<tr>
<td>Fid/Bio Wy. Ratio</td>
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<td>0.053±0.005</td>
<td>0.039±0.007</td>
<td>0.027±0.014***</td>
</tr>
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</table>

*p<0.05, **p<0.01, ***p<0.001 compared to control. N=7-8 per group. Data are mean ± SEM

SLX-4090 dosing levels were also dose-dependently decreased total cholesterol and LDL levels while raising HDL levels.
Dectin-1, a Non-TLR Pattern Recognition Receptor, Regulates NADPH Oxidase Activity in Primary Human Monocytes

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Our lab is interested in studying signal transduction pathways that regulate the activity of NADPH oxidase in primary human monocytes. Zymosan, a yeast cell wall preparation, and its opossum form (ZOP) are potent stimulators of NADPH oxidase activity. The activation of this enzyme complex results in the production of a superoxide anion burst in monocytes. Monocyte-derived superoxide anion mediates LDL lipid oxidation which is believed to contribute to chronic inflammation and specifically atherogenesis. Our lab has previously identified and characterized several pathways that regulate the activity of NADPH oxidase and superoxide anion production in activated monocytes, however, the receptor(s) responsible for zymosan and (ZOP) recognition have not yet been determined in primary human monocytes. We hypothesized that zymosan signals through a pattern recognition receptor for the activation of the NADPH oxidase enzyme complex. Therefore, to test this hypothesis we examined the role of different pattern recognition receptors such as Toll-like receptors (TLRs) and the β-glucan receptor, Dectin-1. Our studies show that zymosan binds to Dectin-1 to trigger a superoxide anion burst in primary human monocytes and this reaction is independent of TLR2 and TLR4. In addition, we found that (ZOP) activates NADPH oxidase through binding to complement receptor 3 (CR3) as well as Dectin-1. We are further trying to link zymosan recognition to intracellular signaling events. Currently, we are investigating receptor phosphorylation and recruitment of intracellular proteins to the activated receptor upon zymosan stimulation. Taken together, our results suggest for the first time that Dectin-1 is the predominant receptor for zymosan-mediated activation of NADPH oxidase in primary human monocytes.

In Vivo Effect of Inflammatory Cytokines on Cystatin C Among Subjects at Risk for Advanced Atherosclerosis

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Background In vitro evidence suggests that inflammatory cytokines augment the secretion of Cystatin C (CysC), and other cell matrix proteases important in atheroma fibrous cap degradation, and coronary plaque rupture. Recently, CysC has been proposed as a method to monitor atheroma fibrous cap degradation, and coronary plaque rupture. Currently, CysC has been proposed as a method to monitor atheroma fibrous cap degradation, and coronary plaque rupture. Therefore, to test this hypothesis we examined the role of different inflammatory cytokines such as TNF-α and IL-6 on CysC secretion in human monocytes, as well as in primary human monocytes. We found that (ZOP) activates NADPH oxidase through binding to complement receptor 3 (CR3) as well as Dectin-1. We are further trying to link zymosan recognition to intracellular signaling events. Currently, we are investigating receptor phosphorylation and recruitment of intracellular proteins to the activated receptor upon zymosan stimulation. Taken together, our results suggest for the first time that Dectin-1 is the predominant receptor for zymosan-mediated activation of NADPH oxidase in primary human monocytes.

Increased Ability of Sera from LCAT-deficient Subjects to Promote ABCA1-mediated Cholesterol Efflux

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Forty-one carriers of mutant LCAT alleles and 10 non carriers from the same families volunteered for this study. In homozygotes (n =14) plasma HDL-C concentration was markedly reduced (11.7±1.7mg/dl) as well as plasma apoA-I (46.2±4.4mg/dl). The analysis of HDL size showed a predominance of small HDL-3 particles, with a great proportion of pre-beta HDL. Heterozygotes (n =27) have slightly reduced HDL-C and apoA-I levels (41.2±2.2mg/dl and 107.0±4.2mg/dl), with a significant increase (39%) in pre-beta HDL. The capacity of serum from LCAT deficient subjects and controls to extract cell cholesterol through the various pathways was tested in different cell models: 1) Fu5AH hepatoma cells, expressing high levels of SR-BI and low levels of ABCA1, 2) parent and mBC01-expressing CHO-K1 cells were used to examine the ABCG1-mediated cholesterol efflux and 3) J774 macrophages expressing high levels of ABCA1, upon treatment with caMP, and low levels of SR-BI. In Fu5AH cells, cholesterol efflux to sera from the homozygotes was significantly reduced by 42% compared with heterozygotes and by 48% compared to control sera with a significant correlation between SR-BI-mediated efflux and HDL-C serum concentration (R =0.698 P<0.001). The ABCG1-mediated cholesterol efflux to LCAT deficient sera was significantly reduced (6.5±0.5% for homozygotes carriers and 8.0±0.2% for heterozygotes) compared to efflux induced by control sera (8.9±0.5%). Under basal conditions, the J774 macrophages release membrane cholesterol to extracellular acceptors mostly by passive diffusion and in such condition, cholesterol efflux to sera from homozygotes carriers was significantly reduced (6.1±0.4%) compared to efflux induced by heterozygotes and control sera, which were similar (9.4±0.4% and 10.5±0.7%, respectively). On the contrary, ABCA1-mediated efflux to the sera was increased by 70% in heterozygotes and 100% in homozygotes subjects compared to control sera, and correlated with the percentage of pre-beta HDL (R =0.480 P<0.001), suggesting that despite the dramatic hypoalphalipoproteinemia, LCAT deficient homozygotes have highly efficient HDL particles in specifically promoting the ABCA1 efflux pathway.
**Telomerase Reverse Transcriptase Is Induced in Macrophages by Proinflammatory Stimuli and Mediates Macrophage Survival and Inflammation**

Florence Gizard, Takashi Nomiyama, Elizabeth B Heywood, Karrie L Jones, Dennis Bresnimmer; University of Kentucky, Lexington, KY

An emerging consensus underscores the importance of both macrophage inflammation and apoptosis in the vascular wall for the development of atherosclerosis. Although the intracellular pathways controlling both processes still remain to be elucidated, a novel area in vascular biology involves telomerase activation. Telomerase controls key cellular functions including replicative lifespan, proliferation, differentiation and apoptosis, and activation of telomerase has been demonstrated in response to injury of the arterial wall. In the present study, we analyzed the role of telomerase in macrophage biology. We demonstrate expression of the catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), in macrophages of human atherosclerotic lesions. Stimulation of primary murine peritoneal macrophages with lipopolysaccharide (LPS) and oxidized LDL (oxDL) resulted in a significant dose-dependent induction of TERT mRNA and protein expression. The up-regulation of TERT in response to LPS or oxDL was prevented by co-treatment with the SNS5 peptide and BAY 11–7082, two inhibitors of the NF-κB pathway. Consistent with these findings, transient transfection experiments using an NF-κB-based reporter indicated a proximal NF-κB site which mediates the transcriptional induction of TERT expression in response to oxDL. Finally, functional experiments demonstrated that TERT-deficient macrophages reveal typical features of cell senescence and were more prone to oxDL-induced apoptosis. Furthermore, oxDL-induced expression of the pro-inflammatory gene IL-1β and the matrix-metalloproteinase-9 (MMP-9) was prevented in peritoneal macrophages isolated from TERT-deficient mice. In conclusion, these results indicate that oxDL induces TERT expression through an NF-κB-dependent pathway and suggest that telomerase mediates macrophage viability and pro-inflammatory gene expression.

**Macrophage j3 Integrin Mediates High-Fat-Diet-Induced Inflammation Through TNFα**

Jochen G Schneider, Yinmu Zhu, Clay F Semenkovich; Washington University, St. Louis, MO

Integrins are critical effectors of inflammatory cell function, chronic treatment with beta3 integrin inhibitors increases mortality in humans, and beta3 integrin deficiency causes atherosclerosis and death in certain high-fat-fed mice. To test the hypothesis that beta3 integrin-deficient (beta3 knockout) mice exhibit improved macrophage accumulation and reduced inflammation in the heart during Western diet feeding, beta3 wild-type mice, 11 of 23 mice transplanted with beta3-deficient marrow survived 12 weeks of Western diet compared to 19 of 21 mice receiving wild type marrow (p < 0.0001). In LDL-deficient beta3 wild-type mice, 11 of 23 mice transplanted with beta3-deficient marrow survived 12 weeks of Western diet compared to 19 of 21 mice receiving wild type marrow (p < 0.0001). LDL-deficient beta3 wild-type mice transplanted with beta3-deficient marrow surviving for 12 weeks on Western diet had no evidence of atherosclerosis that was present in LDL-transplanted mice. To test the hypothesis that beta3 integrin-mediated inflammation of non-cells in the inflammatory phenotype, 19 of 19 beta3-deficient mice in the apoE null model transplanted with beta3 wild-type (apoE null) marrow survived the dietary challenge, compared to 2 of 14 beta3-deficient mice receiving beta3-deficient marrow (p = 0.0007) and a lowering of the quotient oxLDL/LDL by 17.0% (p < 0.0254) after the 2 month medication regimen. Furthermore, we measured a significant decrease in lipopolysaccharide concentration falling from 5.24 ± 8.2 to 42.3 ± 9.9 mg/dL (p < 0.0039). Altogether, these beneficial effects of Ginkgo biloba might have partially repaired endothelial dysfunction being responsible in the early stages in atherosclerosis and could present a basis for a mechanistic explanation of nanoplaque reduction under ginkgo treatment.

**Ginkgo Biloba (EGb 761) Reduces Arteriosclerotic Nanoplaque Formation and Size in Cardiovascular High-risk Patients**

Petta Schäfer, Charité, Campus Benjamin Franklin, Berlin, Germany; Lovisa Ringstad, Uppsala Univ Institute of Pharmacy, Uppsala, Sweden; Sören Just, Heart Center Brandenburg, Germany; Julia Winkler, Univ Clinic Freiburg, Freiburg, Germany; Günter Siegel; Charité, Campus Benjamin Franklin, Berlin, Germany

Utilizing the isolated lipoprotein receptor synedcan (heparan sulfate proteoglycan (HS-PS) from arterial plaque and vascular tissues, we can now measure changes in lipid accumulation and cholesterol content of the biological plaque that can be observed to the very earliest stages of arteriosclerotic plaque development, the so-called nanoplaque build-up, by ellipsometric techniques (patent EP 0 946 876). The arteriosclerotic nanoplaque is represented by the tertiary aggregation complex of the HS-PS receptor, lipoprotein particles and calcium ions. The model was validated in several clinical studies on cardiovascular high-risk patients applying their blood lipoprotein fractions. In eight high-risk patients who had undergone aortic bypass operation bypass, the reduction of arteriosclerotic nanoplaque formation amounted to 11.9% (p < 0.0078) and of nanoplaque size to 24.4% (p < 0.0397) respectively, in normal blood patients soluble with 2.5 mmol/L NaCl after a 2 month therapy with 2 x 120 mg Ginkgo biloba extract (EGb 761, Rikun® novi). Additionally, we could directly demonstrate and confirm the antioxidative capacity of ginkgo and its oxygen free radical scavenging effect by disclosing an upregulation of superoxide dismutase (SOD) activity of 15.79% (beta3 knockout) and a lowering of the quotient oxLDL/LDL by 17.0% (p < 0.0254) after the 2 month medication regimen. Furthermore, we measured a significant decrease in lipopolysaccharide concentration falling from 5.24 ± 8.2 to 42.3 ± 9.9 mg/dL (p < 0.0039). Altogether, these beneficial effects of Ginkgo biloba might have partially repaired endothelial dysfunction being responsible in the early stages in atherosclerosis and could present a basis for a mechanistic explanation of nanoplaque reduction under ginkgo treatment.
was the cause of death and there was marked induction of lung TNFalpha expression. TNFalpha expression was increased 3-fold in peritoneal macrophages of mice transplanted with beta3-deficient marrow as compared to wild type-transplanted mice (p < 0.05), an effect prevented by treatment with an anti-TNFalpha antibody but not with an isotype-specific control antibody (p < 0.01). These data suggest that macrophage beta3 integrin modulates dietary inflammation through TNFalpha.

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Cosegregation of Sca1 Mutations with Dyslipidemia, Body Weight, and Atherosclerosis in an Intercross Between C57BL/6 and C3H Apolipoprotein E-Deficient Mice

Weibin Shi, Toru Miyoshi, Zuobiao Yuan, Timothy R Gilbert; Univ of Virginia, Charlottesville, VA

Background: Dyslipidemia is an integral component of the metabolic perturbations in several common human disorders, including type 2 diabetes, obesity, and the metabolic syndrome. Genetic factors are a major determinant for the pathogenesis of dyslipidemia. Sterol o-acetyltransferase 1 (Sca1), also known as ACAT1, is an endoplasmic reticulum enzyme that catalyzes free cholesterol to cholesteryl ester. Methods and Results: We identified four single-nucleotide polymorphisms (A421C, A439G, C454T, and C613T) within the coding region of Sca1 between C57BL/6 (B6) and C3H mice and two of the SNPs led to amino-acid substitutions (Ile147Val and His2057yr). In an intercross between B6 and C3H apolipoprotein E-deficient (apoE-/-) mice, allele variation at Sca1 (for a closely-linked gene) explained 13% to 16% of the variations in plasma triglyceride, HDL, non-HDL cholesterol levels, body weight, or atherosclerotic lesions. Inheritance of the C3H alleles resulted in significant increases in plasma lipid levels and body weight but decreases in atherosclerotic lesion size compared to inheritance of the B6 alleles. All associations with variation in plasma lipids, body weight, or atherosclerotic lesions, as well as in plasma HDL and non-HDL cholesterol levels and body weight, were maintained in both male and female mice. Conclusion: The close link between the C3H alleles and atherosclerotic lesions suggests that Sca1, or a closely linked gene, can contribute to dyslipidemia and atherosclerosis.

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Microarray Gene Expression Profiling Identifies Candidate Atherosclerosis Modifier Gene for the Ath26 Locus in Male Apo-E-Deficient Mice

Jonathan D Smith, Enakshi Chakrabarti, Jeffrey M Bhasin; Cleveland Clinic Lerner College of Medicine, Cleveland, OH

Objective-To identify genes that modify atherosclerosis severity in a mouse model. Methods and Results- In a strain intercross between atherosclerosis resistant AKR apoE-deficient mice and atherosclerosis sensitive DBA2 apoE-deficient mice, we identified a quantitative trait locus (QTL) on chromosomes 17 that is associated with lesion severity in the male F2 cohort. This QTL, called Ath26, at 34 Mb on chromosome 17, has a LOD score of 4.25, with a genome wide significance of p < 0.05. The AKR locus at this QTL has a dominant effect on lesion area, with the DBA2 allele associated with increased lesion severity, and, variation at this one locus is associated with 14.5% of the lesion variation in the male cohort. Bone marrow derived macrophages were cultured from 114 male F2 mice, and mRNA was prepared and used for expression level of a specific transcript. More than 12,300 eQTLs were identified with mapping cis to the gene (cis eQTL) and the majority mapping trans to the gene (trans eQTL). We then performed correlation analyses to identify the genes whose expression was best associated with lesion severity. The majority of these genes, over 95%, were found within 150,000 base pairs from the gene. Conclusion: This is the first study to identify a candidate atherosclerosis modifier gene for the ath26 locus. This candidate gene, Sca1, which is abundantly expressed in arterial walls, is an integral component of the metabolic perturbations in several common human disorders. Apolipoprotein B-100 (apoB) is important for assembly of VLDL in liver and the clearance of VLDL and LDL particles from plasma. Reduction of apoB mRNA expression is known to lower the secretion of VLDL from the liver and reduce plasma cholesterol thereby and the risk of developing atherosclerosis. We report on the significant locked nucleic acid whose effect on a region from CAST which contains an HDL gene.

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Loss of BMPII in Endothelium Induces Inflammation in Vitro and Correlates with Atherosclerosis Progression in Human Coronary Arteries

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Atherosclerosis is an inflammatory disease, occurring preferentially in arterial regions associated with disturbed flow while sparing the undisturbed flow regions. We have shown that exposure of endothelial cells (ECs) to disturbed flow stimulates production of bone morphogenic protein receptor (BMPR) II (or BMPRII), which leads to inflammatory responses - intercellular adhesion molecule-1 (ICAM-1) expression and subsequent monocyte adhesion. However, the underlying mechanism by which BMPR II induces inflammation is unclear. Here, we examined which BMP receptors (BMPR) mediate BMPR IV action in ECs. Studies using mouse aortic ECs (MAEC), human umbilical vein ECs (HUVEC), mouse thoracic aorta and human coronary arteries revealed that BMPR II (ALK2) and BMPR RII (ALK3) were expressed in ECs. Interestingly, immunostaining studies showed that BMPR II was located in the cell-cell junction, colocalizing with VE-cadherin, in confluent MAEC but not in ECs that were sub- confluent or at wounded edges. MAEC treated with mouse-specific siRNA lost BMPR II expression in the cell-cell junction. The BMPR II knockdown prevented phosphorylation of smad1/5/8 and stimulation of monocyte adhesion in response to BMP4, suggesting a role for BMPRII as the BMP receptor. Unexpectedly, BMPRII knockdown in ECs also attenuated the inflammatory response in the absence of BMP4 as determined by increased ICAM-1 expression and monocyte adhesion, which was blocked by an ICAM-1 neutralizing antibody (Y11). Moreover, BMPRII levels in ECs were progressively decreased in more advanced atherosclerotic lesions in human coronary arteries. These findings suggest that a decrease in BMPR II expression in ECs induces inflammation and is associated with more advanced atherosclerotic disease states.

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Arsenic-Induced Activation of Atherogenesis in Apo-knockout Mice: Role of Oxidative Stress and Inflammation

Sanjay Srivastava, Stanley E D’Souza; J C States; Univ of Louisville, Louisville, KY

Chronic arsenic (As) ingestion causes inflammation and vascular dysfunction in humans. In the present study, we examined the effect of As-feeding on atherogenesis in apoe-knockout mice. Three-week old normal mice were fed on normal chow with or without water (n = 14) or water containing 1 (n = 12), 4.9 (n = 12) or 49 (n = 18) ppm As. The mice were sacrificed at age 16 weeks of age and atherosclerotic lesion formation, plasma lipids and indices of oxidative stress and inflammation were examined. Plasma cholesterol and phospholipids of the As-fed mice were comparable to the controls. Plasma triglycerides of the As-fed mice were increased 1.5-fold above normal control levels. Inflammatory cytokines and chemokines (TNF-alpha, IL-1beta, MCP-1, VEGF and lipid peroxidation products malondialdehyde (MDA) and 4-hydroxy-trans-2-nonenal (HNE)) were significantly increased (P < 0.05) and throughout the aortic tree at 36 weeks of age (n = 12; P < 0.05). Inflammatory cytokines and chemokines in these mice showed a 2-fold increase in the macrophage accumulation in the aortic valve, whereas very little if any T-cells or smooth muscle cells were detected in these lesions. These mice also showed increased staining for the markers of inflammation (MCP-1) and oxides stress (protein-HNE and protein-MDA) in the aortic valve. Collectively, our data suggest that exposure to As enhances oxidative stress and inflammation and accelerates atherogenesis in apoe-knockout mice.
polymorphisms that changed amino acid. These changes are Leu169Phe in gene 
encoding enoyl-CoA hydratase protein band 4.9, Asn245Ser in Xpo7 encoding exportin 7, Thr911Met in Dock2 encoding docking protein 2, and Thr915Ala in gene Fndc3a encoding fibronectin type III domain containing 3a. None of these changes alter the acidity of amino acid, but two do change the amino acid from polar to non-polar (Thr/Ala in Fndc3a and Thr/Met in Dock2). Further evidence is needed to determine which of these is the gene affecting HDL.

**The Hemodynamic Environment Promotes Atherosclerosis-prone Phenotypes in Endothelial and Smooth Muscle Cells via a Mechanical-Transcription Coupling Mechanism**

Nicole Hastings, Michael Simmers, Brian Wambhoff, Brett Blackman; Univ of Virginia, Charlottesville, VA

**Introduction** Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where athero-prone flow leads to increased susceptibility to atherosclerosis. The internal carotid sinus (ICS) is distinguished by its low disturbed flow profile (athero-prone) and is thus a common site of atherosclerotic lesion formation, whereas the common carotid artery (CCA) is exposed to pulsatile laminar flow (athero-protective). Hypothesis A novel in vitro co-culture model has been developed to analyze the mechanical-transcription coupling that occurs in human endothelial cells (EC) in contact with human smooth muscle cells (SMC) during athero-prone hemodynamics. Here, we tested the hypothesis that athero-prone flow applied to ECs induces SMC and EC phenotypes characteristic of early atherosclerosis. Methods Human ECs/SMCs were plated on the top and bottom surface of porous transwell membranes (75nm) and grown to confluence. Hemodynamic flow patterns derived from human MRI images of the ICS and CCA were applied to the EC surface for 24 hours using a cone and plate flow device. Results/Conclusions ECs display decreased alignment and elongation in response to athero-prone compared to protective flow, while SMOs were oriented perpendicular to flow only during athero-prone flow; both reminiscent of in vivo architectures. Gene expression analysis confirms the presence of an SMC athero-prone phenotype defined by reduction of SMC markers (smooth muscle α-actin, myocardin) and increased levels of transcription factor KLF4 and inflammatory gene VCAM-1, resulting from the flow regime applied to ECs. Also, athero-prone-exposed ECs exhibited a reduction in Tie2, eNOS and KLF2 gene expression and discontinuous staining pattern of VE-cadherin, suggestive of a pro-remodeling state, loss of protective effects, and a more porous permeability barrier. Importantly, the chemokine interleukin-8 is upregulated in ECs exposed to athero-prone flow, which may potentiate part of the SMC athero-prone phenotype. This study indicates that SMCs undergo differential phenotypic changes in athero-prone flow dependent upon EC mechanico coupling, leading to a priming effect reflecting early atherosclerosis of the arteries.

**Combined Deficiency of αt(1,3)-fucosyltransferase-IV and -VII Is Required to Limit Atherosclerosis in Mice Lacking the Low-density Lipoprotein Receptor**

Jonathan Homeister, Maria Morales-Levy; Univ of North Carolina, Chapel Hill, NC

Atherosclerosis is due to an inflammatory/immune response that requires leukocyte recruit- ment to the vessel wall, mediated by E- and/or P-selectin and other leucocyte adhesion molecules. αt(1,3)-fucosyltransferase (FucT)-IV and FucT-VII synthesize active fucosylated selectin ligands. To determine a role for these enzymes in the atherosclerotic disease process, this study tests the hypothesis that deficiency of FucT-IV and/or FucT-VII will decrease aortic root lesion size in the low density lipoprotein receptor (LDLR) deficient mouse model of atherosclerosis. Mice deficient in FucT-IV and/or FucT-VII were crossed onto the LDLR(-/-) strain and fed a normal chow diet for six months. Aortic root lesion size was determined by planimetry, plasma cholesterol and triglyceride concentrations by colorimetric assay, and blood cell counts by automated blood analyzer. Cholesterol and triglyceride lipoprotein distribution profiles were determined by FPLC. Lesion size [mm²; mean ± sem; n=5] in male mice was decreased in FucT-IV(-/-)/FucT-VII(-/-)/LDLR(-/-) animals [0.02 ± 0.003; 5], as compared to FucT-IV(-/-)/LDLR(-/-) [0.09; 0.006; 5]. In female mice, compared to FucT-IV(-/-)/LDLR(-/-) [0.02 ± 0.001], lesion size in female mice was not significantly different in FucT-VII(-/-)/LDLR(-/-) [0.02 ± 0.03; 12] or FucT-IV(-/-)/FucT-VII(-/-)/LDLR(-/-) mice [0.13 ± 0.07; 12], compared to control LDLR(-/-) mice [0.17 ± 0.03, 12]. Cholesterol or triglyceride distribution among lipoprotein fractions was not genotype-dependent. Significant changes in total plasma cholesterol or triglyceride, when present, did not correlate with the observed genotype-dependent changes in lesion size. FucT-IV(-/-)/FucT-VII(-/-)/LDLR(-/-) and FucT-IV(-/-)/FucT-VII(-/-)/LDLR(-/-) mice had a monolayer (2.7–4.7 fold), a lymphocytosis (1.5–2.7 fold), and a granulocytosis (4.0–11.0 fold), compared to LDLR(-/-) mice. These results show that loss of selectin ligand activity achieved by deletion of both FucT-IV and FucT-VII results in decreased aortic root lesion size in male LDLR(-/-) mice, despite the presence of a significant leukocytosis.

**Does Elevated Macrophage uPA Expression Cause Plaque Rupture in Advanced Atherosclerotic Lesions?**

Jie Hu He, Nagadha Dranadula, Gore Otsuka, David A Dichek; Univ of Washington, Seattle, WA

Background: The cellular and molecular mechanisms of athero-prone plaque rupture are poorly understood. Increased proteolytic activity of lesion macrophages is often proposed as a cause of plaque rupture. Understanding the role of the protease uPA in macrophage transmigration, plasminogen activation and proteolysis of multi-layered atherosclerotic plaques, is expressed in human atherosclerotic lesions, primarily by macrophages. uPA/plasminogen can activate matrix metalloproteinases, which have been implicated as causes of plaque rupture. Hypothesis: We hypothesized that overexpression of uPA by macrophages in advanced lesions of apolipoprotein E null (apoE(-/-)) mice will cause plaque rupture. Methods: Bone marrow from transgenic mice with macrophage-targeted uPA overexpression (sr-uPA(+) mice) or nontransgenic donors (sr-uPA(-/-) mice) was transplanted into irradiated 36-wk-old SR-APoE(-/-) recipients. Some of the donor mice were also transgenic for GFP. Recipient mice were euthanized at 7 wk after transplantation. The aortas were collected and fixed. Lesion histology was examined at 12 wks by GFP immunostaining to determine whether donor-derived cells were present, and by qRT-PCR at 8–10 wks to measure uPA expression. Results: A low analysis demonstrated adequate BM cell reconstitution by deletion of both FucT-IV and FucT-VII results in decreased aortic root lesion size in male LDLR(-/-) mice, despite the presence of a significant leukocytosis.

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sections showed that numerous donor-derived macrophages were present in innominate artery lesions. TaqMan rT-PCR showed significantly higher expression of uPA mRNA in innominate arteries from recipients of SR-uPA-BM than from innommates from recipients of SR-uPA-BM (relative uPA expression: 2.1 ± 0.31 vs 6.0 ± 0.038 arbitrary units; p = 0.007; n = 8). Conclusion: We introduced uPA-overexpressing macrophages into advanced innominate artery lesions of Apoe-/- mice. These macrophages express high levels of uPA after 10 d in the lesions, resulting in a large (20-fold) increase in lesion uPA expression. This experimental setting will allow us to test whether elevated macrophage-targeted uPA expression in innominate lesions precipitates plaque rupture.

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Antithrombotic and Anti-inflammatory Effects of Polyphenol-enriched Fraction from Taraxacum coreanum Nakai in LDLR-Deficient Mice
Jong-Min Han, Yong-Dae Park, Sojin An, Min-Jung Kim, Yue-Jan Jin, Woo S Lee, Tea-Sook Jeong; KRBIB, Daejeon, Republic of Korea

There have been numerous reports demonstrating antithrombotic activity of polyphenols. Natural polyphenols have a wide range of biological activities. The polyphenol-enriched fraction (PEF) isolated from dried whole plants of Taraxacum coreanum Nakai decreased nuclear factor-κB (NF-κB) activation. The PEF contains many biologically active polyphenols, luteolin (1.28%), luteolin-7,7′-digenin (0.2%), caffeic acid (0.15%), apigenin (0.15%), and quercetin (0.09%), being the major constituents. In this study, we investigated early stages antithrombotic effects of the PEF in a high-cholesterol diet-fed LDL receptor deficient (LDLR-) mice. At 10 weeks of age, 20 male LDLR-/- mice were randomly divided into two groups (n = 10) and fed a high-cholesterol diet (0.15% w/v/diet, control group) or a high-cholesterol diet supplemented with PEF (0.2% w/v/diet) for 8 weeks. There were no differences in total cholesterol and body weight between the control group and PEF-supplemented group during the study period. However, the triglyceride level was decreased in the PEF-supplemented group. The-a-sialation of cholesterol roots also significantly reduced the a-sialation in lesion areas. The mean lesion areas of 10 consecutive sections stained with oil red O were 56.2 ± 29.7 μm²×10⁻³ in the PEF-supplemented group versus 93.8 ± 38.1 μm²×10⁻³ in control group (p > 0.05). The PE protein levels which in turn regulated by NF-κB, such as VCAM-1, ICAM-1, TNF-α, and COX-2, were suppressed in aorta. In addition, the PEF significantly suppressed the production of nitric oxide (NO) and the accumulation of intracellular reactive oxygen species (ROS) in a dose-dependent manner by inhibiting NF-κB activation in LPS-induced RAW 264.7 cells. In conclusion, our results suggest that the PEF containing luteolin, luteolin-7,7′-diglucine, caffeic acid, apigenin, and diosmetin may be a promising product for treatment of atherosclerosis by inhibiting NF-κB and providing additional rationale for application of anti-inflammatory therapeutic approaches for atherosclerosis prevention.

Downregulation of Tissue Inhibitor of Metalloproteinases Increases Invasion, Proliferation, and Death of Macrophage-derived Foam Cells
Jason L Johnson, Andrew C Newby; Univ of Bristol, Bristol, United Kingdom

Foam cell macrophages secrete higher levels of matrix metalloproteinases (MMPs) than non-foamy macrophages which contributes to plaque development and rupture. We investigated the expression of the endogenous tissue inhibitor of metalloproteinases (TIMP)-3 during foam cell formation, and its effects on macrophage and foam cell behaviour. Foam cells derived from cholesterol-fed rabbits and by Ox-LDL loading of human macrophages demonstrated a significant decrease in TIMP-3 mRNA (55%, p = 0.01 and 49%; p = 0.05, respectively) and protein production (84%, p = 0.003) compared to control macrophages. Adding back TIMP-3 to foam cells significantly inhibited migration (51%, p < 0.05), proliferation (70%; p < 0.05) and apoptosis (36%; p < 0.05), but had no effect on non-foamy macrophages. Immunocytochemistry for TIMP-3 on foam cells revealed a subset of these cells (28%) that were TIMP-3 negative. Furthermore, only cells negative for TIMP-3 invaded a synthetic basement membrane using an in vitro invasion assay. In early rabbit atherosclerotic plaques, TIMP-3 expression was negative. Furthermore, only cells negative for TIMP-3 invaded a synthetic basement membrane. This study demonstrates that TIMP-3 is essential for controlling macrophage migration and proliferation in early atheroma. The loss or reduction of TIMP-3 expression may be a potential therapeutic target.

Liver X Receptors Enhance Apolipoprotein A-1 and Paraoxonase-1 Expression in Mouse Hepatocytes and Prevent the NF-κB Induced Alterations in apoA-1, PON-1, and SAA in Inflammatory States
Atil Y Kargi, Chang Yeop Han, Mohamed A Omer, Alan Chait; Univ of Washington, Seattle, WA

Atherosclerosis is a disorder of lipid metabolism and an inflammatory disease. Liver X receptors (LXR) reciprocally regulate inflammation and lipid metabolism in macrophages. LXRs increase expression of several genes involved in reverse cholesterol transport including ABCA1, ABCG1, ABCG5, ABCG8, ATP5, apoA, and CPT1A1. However, little is known about the role of LXRs in regulating the hepatic expression of apoA-1, a key acceptor in reverse cholesterol transport. LXRs regulate inflammatory gene expression via the antagonism of NF-κB signaling. In hepatocytes, we have shown that NF-κB activation upregulates SAA expression and decreases paraoxonase-1 (PON-1) and apoA-1 production, changes which might decrease the antiatherogenic effects of increased NMDA-receptor (NMDA) activation in the brain. In the current study, we found that apoA-1 and PON-1 expression while inhibiting the effects of inflammatory cytokines on SAA, apoA-1 and PON-1. To test this hypothesis, we measured the effect of the endogenous LXR ligand 22R-hydroxysterol (22R-H) and T0901317, a potent and specific synthetic LXR ligand, on SAA, apoA-1 and PON-1 expression in cultured mouse AML12 hepatocytes. Both 22R-H and T0901317 increased hepatic apoA-1 and PON-1 expression as much as 4 fold (p < 0.05) while SAA expression remained very low. Activation of LXR 4 h prior to treatment with either LXR agonist induced 95% and 90% respectively decrease in NF-κB prevented the cytokine-mediated increase in SAA and decreases in apoA-1 and PON-1. These LXR effects occurred at concentrations as low as 100 nM and peaked at 10 μM for T0901317 and between 10 μM and 50 μM for 22R-H. Thus we have shown that LXRs, in addition to their well-known effects de novo genes regulate inflammatory and cholesterol transport hepatic expression of apoA-1, the major acceptor protein in reverse cholesterol transport. Moreover, by inhibiting the effects of inflammatory cytokines on apoA-1, PON-1 and SAA, LXRs also have an anti-inflammatory effect on HDL apolipoproteins. This reciprocal regulation of lipid metabolism and inflammation at the level of the liver could result in a more atheroprotective HDL particle, which suggests a novel mechanism by which LXRs might prevent atherosclerosis.

Is Coronary Calciumification Protective of ST-elevation Myocardial Infarction?
Shazib Khawaja, Nadeem Husain, Malik Ali, Hiraan Ahmed, Asmir Syed, Frank Petryjohn; Univ of South Alabama, Mobile, AL

Background: The association between coronary calcification and acute coronary syndromes (ACS) especially ST-elevation myocardial infarction (STEMI) has not been extensively studied. It has been suggested that plaques containing calcium may be less vulnerable to rupture. We studied the extent of coronary calcification as assessed by coronary angiography in the infarct related arteries among patients with STEMI in comparison with patients with chronic angina requiring revascularization. We hypothesized that the presence of coronary calcification may be protective for STEMI.

Methods: Coronary calcification, as assessed by angiography, was graded as none, mild, moderate or severe at the site of lesion extending 5mm proximal and distal to the culprit lesion in patients with STEMI and chronic angina. Results: 50 patients with STEMI as well as 50 consecutive patients with stable angina in a non ACS setting presenting for outpatient coronary angiography and receiving PCI or CABG for severe (≥ 30%) stenosis were studied. 72% of the patients with STEMI had no angiographic calcification in comparison with only 10% of the patients with chronic angina requiring revascularization. Overall there were more patients with moderate and severe calcification in the chronic angina arm than the patients with STEMI. Conclusion: In conclusion coronary calcification is noted to be lower in culprit lesion among patients with STEMI as opposed to patients with chronic coronary atherosclerosis. The low prevalence of coronary calcification with STEMI supports the possibility of calcium as protective in preventing plaque rupture.

Patient Characteristics

<table>
<thead>
<tr>
<th>STEMI (n=50)</th>
<th>Chronic Angina (n=50)</th>
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<tbody>
<tr>
<td>Age(years)</td>
<td>69.3 ± 11.2</td>
</tr>
<tr>
<td>Sex(M/F)</td>
<td>39/11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>35(70%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>32(64%)</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td>33(66%)</td>
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<tr>
<td>Results</td>
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Conclusion: In conclusion coronary calcification is noted to be lower in culprit lesion among patients with STEMI as opposed to patients with chronic coronary atherosclerosis. The low prevalence of coronary calcification with STEMI supports the possibility of calcium as protective in preventing plaque rupture.
High-density Lipoprotein Cholesterol Response to Statin Therapy in Korean Hypercholesterolemic Patients

Min-Kyung Kim, Yeon-Yee Yoon, Seoul National Univ Hosp, Seoul, Republic of Korea; Sang-Hyun Kim, Joo-Hee Jo, Myung-A Kim, Seoul Metropolitan Baramae Hosp, Seoul, Republic of Korea; Dong-Ju Choi, Seoul National Univ Bundang Hosp, Sungnam, Republic of Korea; Hyo-soo Kim, Dae-Won Sohn, Byung-Hee Oh, Young-Bae Park, Yun-Sik Choi; Seoul National Univ Hosp, Seoul, Republic of Korea

Background and aims: Statins generally increase high-density lipoprotein cholesterol (HDL-C), but not in all patients. We designed a retrospective study 1) to assess the characteristics and the different response patterns of lipoprotein level after statin therapy, comparing the patients with decreased HDL-C level after statin therapy (poor response group) and the patients with preserved or increased HDL-C level after statin therapy (favorable response group), 2) to investigate the problems of statin-only-strategies in hypercholesterolemia especially in the patients with high risk. Methods: 516 patients, who were newly diagnosed as hypercholesterolemia, were enrolled. Their clinical characteristics, baseline and follow-up laboratory data were analyzed. All patients had received one kind of statins for at least 6 months. The ‘poor response group’ showed more decrease of TC (p<0.001) and LDL-C (p<0.001) and increase of TG (p<0.001) than the favorable response group. Patients with initial low HDL-C level showed poor responses to statins in terms of TC (p=0.011) but better responses in terms of HDL-C than those with normal initial HDL-C level (HDL-C increased by 11.4% vs. 2.2% p<0.001). Conclusions: Serum HDL-C and TG level increased with statin therapy in some patients, but decreased in the others. The risk of HDL-C decrease with statin therapy coexists with large reduction of LDL-C level, so HDL-C raising therapy can be considered with statin therapy especially in the patients of high risk group.

Newly Developed PPAR-α Agonist (R)-K-13675 Inhibits the Secretion of Inflammatory Markers from Human Coronary Artery Endothelial Cells

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Peroxisome proliferator-activated receptor-alpha (PPAR-α) is a key regulator of lipid and glucose metabolism and implicated in inflammation. (R)-K-13675 is a novel potent PPAR-α agonist with high subtype selectivity. Monocyte chemoattractant protein-1 (MCP-1) plays a key role in the early stage of atherosclerosis. Therefore, we analyzed whether (R)-K-13675 decreases MCP-1 secretion and other inflammatory markers such as interleukin-6 (IL-6) and interferon-gamma (INF-γ) in human coronary endothelial cells (HCECs). HCECs were maintained in different doses of (R)-K-13675 under serum starvation. After 20 hours, the levels of MCP-1, IL-6 and INF-γ secretion in the medium were analyzed using ELISA. Treatment with KPA-73979 at 0, 10, 50 and 100 nM, MCP-1 levels were significantly suppressed in dose dependent manner (p<0.05, 2.1–0.1, 1.9–0.1 and 1.3–0.2 pm, respectively). Level of INF-γ and INF-γ levels also significantly suppressed (IL-6: 196.8±194.5, 157.5±194.4pm, 94.1±94.8 pm, respectively; INF-γ: 37±2, 33.3±2, 21.3±2 and 5.3±1.0 pm, respectively). In addition, (R)-K-13675 did not affect HCECs proliferation and tube formation in all doses. Thus, (R)-K-13675 was associated with inhibition of inflammatory responses without affecting of proliferation and angiogenesis, and may induce anti-atherosclerosis.
Cardiovascular disease is currently the leading cause of death in the US, with atherosclerosis being responsible for approximately half of all CVD-related deaths. Epidemiological studies have suggested an association between environmental air pollution and increased rates of cardiovascular morbidity and mortality. We have previously reported that subchronic exposure to gasoline engine emissions results in increased aortic expression of several matrix metalloproteinases (MMPs), which are markers of vascular remodeling and inflammation, in atherosclerotic-prone ApoE-/- mice. However, the underlying molecular mechanisms have not yet been elucidated. We investigated the hypothesis that acute exposure to gasoline engine emissions results in increased levels of vascular reactive oxygen species (ROS), which in turn drive the subsequent induction of MMPs and related peptides such as endogenous tissue inhibitors of MMPs, TIMPs, and endothelin-1 (ET-1). To this end, male ApoE-/- mice (n=6/group), on a high fat diet, were treated orally with either 10 mmol/L of the SOD mimetic, Tempol (avg. 41 mg/kg/day, beginning 24 h prior to exposure, or vehicle), and exposed via inhalation for 6 h/d for a period of 1, 3, 7, or 10 days, either filtered air or gasoline exhaust (a 1:12 mixture of filtered air to gasoline engine emissions results in increased vascular oxidative stress by 27% of exposure to gasoline emission, which was ameliorated with Tempol-treatment. Concurrent significant elevations in aorta MMP-2 and -9 (d7) and TIMP2 (d1) mRNA expression were also observed, each of which was attenuated by Tempol-treatment. Interestingly, aorta ET-1 mRNA was significantly elevated by exposure d3, however Tempol had no obvious effects on expression. Such findings suggest that exposure to gasoline engine emissions results in increased vascular oxidative stress which mediates, at least in part, expression of markers involved in vascular remodeling, inflammation, and the progression of atherosclerosis.

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**Conclusion:**

KCa3.1 Chronic KCa3.1 blockade significantly prevented development of atherosclerosis in EKO by 40% in conjunction with 60-% reduction of macrophage accumulation in the lesions (macrophage-positive area; 81 ± 12 x 10^6 μm², p<0.05 vs control 219 ± 20, n=9). Migratory response to MCP-1 was impaired also in macrophages collected from EKO chronically-treated with TRAM-34 (1.5±0.1-fold of control, p<0.05 vs vehicle 2.3±0.4-fold, n=6). Long-term treatment of EKO with TRAM-34 did have no obvious pathological or clinical signs of toxicity, while plasma TRAM-34 concentrations were maintained at ranges specific to KCa3.1 (866±4.8×10^−6 M, n=6).

**In conclusion:** KCa3.1 activity is associated with macrophage accumulation in atherosclerosis, suggesting a pathophysiological role for KCa3.1 up-regulation in atherosclerosis. KCa3.1 blockade is a new therapeutic strategy for diseases involving activated macrophages such as atherosclerosis.
Contrast-Enhanced Ultrasound Imaging of Atherosclerotic Carotid Plaque Neovascularization: A New Surrogate Marker of Atherosclerosis?

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Objectives: To demonstrate that contrast enhanced carotid ultrasound (CEUS) constitutes an effective method for the detection of carotid intra-plaque neovascularization. Background: An atherosclerotic plaque requires a nutrient blood supply, which is predominantly derived from arterial trunk. A variety of factors (environmental and genetic) contribute to the initiation and growth of atherosclerosis within vessel walls. Chemoattractant factors, such as tissue ischemic and hypoxic factors, stimulate the release of vascular endothelial growth factor (VEGF) proteins, resulting in vessel wall angiogenesis. These developments often precede the formation of the luminal plaque. In this report, we describe the use of contrast enhanced carotid ultrasound (CEU) imaging for the detection and quantification of intra-plaque neovascularization. The efficacy of CEUS was measured against the neovascular density observed within the tissue specimens obtained at the time of carotid endarterectomy surgery. Methods: Fifteen patients with significant carotid stenosis who underwent cervical carotid endarterectomy were studied. Carotid artery ultrasound (CEU) examination prior to undergoing a carotid endarterectomy (CEA). Two patients received bilateral endarterectomies, resulting in a total of 17 cases. At the time of surgery, carotid plaque samples were surgically removed and stained with specific vascular endothelial (CD31), von Willebrand factor, and hemoglobin) designed to identify the presence and degree of neovascularization. The intra-plaque neovascularization recorded on pre-operative CEU was correlated with the degree of neovascularization noted in the tissue specimens. Results: The contrast-enhanced carotid ultrasound (CEU) neovascularization was correlated to EC-31 stained tissue specimens. This correlation value was 0.68 using Spearman’s rank method. Conclusions: The ability of CEU to detect neovascularization appeared to correlate with the presence and degree of intra-plaque neovascularization observed by histologic comparison.

Cyclic Strain Regulates the Notch/CBF-1 Signaling Pathway in Endothelial Cells: Role in Angiogenesis

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The role of hemodynamic forces, such as pressure-induced cyclic stretch, in the control of angiogenesis remains poorly understood. The Notch signaling pathway regulates cell-cell interactions of a variety of cell types. Notch receptors and target genes are upregulated following vascular injury and are reportedly expressed on vascular endothelial cells (EC). We assessed the hypothesis that Notch signaling mediates the cyclic strain-induced angiogenic response of EC. A Flexcell system was used to expose human umbilical vein endothelial cells (HUVECs) to physiological levels of cyclic strain (0–10% strain, 60 cycles/min, 0–24 h). Notch receptor and Notch target gene mRNA and protein levels were determined by QRT-PCR and Western blot analysis, respectively. Network formation on Matrigel was measured as an index of angiogenesis. Exposure of EC to cyclic strain (10%) resulted in temporal regulation of Notch receptors 1 and 4 and target genes (hr1,2,3) at the mRNA and protein level: maximal increase at 4h, 21±8 and 23±9 fold increase, respectively for Notch 1 and 4 mRNA (n=3,3). Moreover, cyclic strain (10%, 24h) significantly increased EC angiogenesis; network length (AU) 775±127 vs 3928±400 for static and strained EC, respectively (n=3–5, p<0.05), while concurrently increasing mRNA expression of the growth factor Angiopoietin-1 (Ang-1) and its receptor tyrosine kinase Tie-2 by 7 and 3 fold, respectively. Knockdown of Notch 1 and 4 by siRNA, or inhibition of Notch mediated CBF-1/RBP-Jk regulated gene expression by RMPMS-1, resulted in a significant decrease in cyclic strain-induced network formation and in Tie2 mRNA-expression. Notch 1 or Notch 4 siRNA, but not RMPMS1, inhibited cyclic strain-induced Ang1. Constitutive over expression of Notch 4 IC resulted in increased network formation, and Ang1 and Tie2 mRNA expression, under both static and strain conditions. These data demonstrate that (i) the Notch signaling pathway in EC is biologically sensitive and (ii) that cyclic strain stimulates angiogenesis is mediated, in part, through a Notch-dependent, Ang-1/Tie2 signaling pathway. Mechanosensitive Notch signaling may thus represent a novel therapeutic target for diseases involving angiogenesis.

Triglyceride-rich Lipoprotein Lipolysis Products Induce Endothelial Cell Inflammation That Is Mediated by Oxidized Lipids

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Objectives: Triglyceride-rich lipoprotein (TRL) lipolysis products provide a pro-inflammatory stimulus to the endothelium. We investigated the mechanism by which TRL lipolysis products induce endothelial cell dysfunction by examining individual lipid fractions. Methods and Results: Lipid classes from human postprandial TRL with or without lipoprotein lipase (LPL) were extracted and separated using amino-propyl solid phase extraction columns. The amount of free fatty acids (FFA) released from TRL by lipoprotein lipase was 10-fold greater than that found in untreated TRL. The FFA extracted from TRL lipolysis significantly increased the expression of superantigen TNFα in human aortic endothelial cells. Reactive oxygen species (ROS) were measured by determining dichlorofluorescin diacetate fluorescence. Treatment with both the FFA and cholesterol fractions caused ROS production; however, the FFA fraction released from TRL lipolysis doubled the production of ROS compared with that released from TRL only. The FFA-mediated increase in ROS was blocked by the cytochrome P450 2C9 inhibitor sulfaphenazole. Further, the levels of oxidized lipids within the FA fraction were determined by HPLC/CMS/MS. Three 3-oxo acyl-CoA dehydrogenase (3-oxo-ACD) metabolites 13-HODE (1.68-fold) and HODE (1.7-fold) and 15-HODE (1.51-fold) were found in TRL lipolysis group. Compared with linoleic acid, 13-HODE significantly induced ROS production in human endothelial cells (1.56-fold). Summary and Conclusions: The pro-inflammatory effect of TRL lipolysis products is mediated, in part, by the free fatty acid fraction. Further more, the linoleic acid derived alcohols, 13-HODE and 9-HODE are released from TRL by LPL and may facilitate endothelial cell injury in vivo by stimulating intracellular ROS production.

A Novel ENOS-independent Protective Action of Statin Against Angiotensin II-induced Cardiovascular Remodeling and Renal Injury

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Background: Statins are known to have pleiotropic actions mainly through activation and up-regulation of the eNOS system. However, the effect of statins on cardiac-renal insufficiency caused by renin-angiotensin activation and the mechanisms of their actions are not fully understood. Methods and Results: We used pitavastatin because this statin is not metabolized by hepatic cytochrome P450, implying that pitavastatin has direct action on the cardiovascular and renal systems. C57BL/6J mice at 10 weeks of age were infused with angiotensin II (Ang II) (2.0 mg/kg/day) by an osmotic mini-pump for 2 weeks and were simultaneously administered pitavastatin (0.2 mg/kg/day) or a vehicle. Echocardiography showed that pitavastatin treatment improved Angi-II-induced left ventricular concentric hypertrophy and diastolic dysfunction. Enhancement of perivascular fibrosis and medial thickening of the coronary artery, of cardiac fibrosis, and of cardiomyocyte hypertrophy by Ang II infusion were significantly attenuated in pitavastatin-treated mice. Pitavastatin treatment also reduced cardiac mRNA expression of p65/70phox and urinary excretion of B-H2dli, an oxidative stress marker. Pitavastatin induced phosphorylated Erk, Akt, JNK, p38 MAPK, TGF-β and Smad 2/3 phosphorylation were all attenuated by pitavastatin treatment. Even in eNOS KO mice, pitavastatin improved Ang II-induced cardiovascular remodeling and left ventricular diastolic dysfunction. In addition, low glomerular filtration rate and enhancement of albuminuria in Ang II-treated eNOS KO mice were improved by pitavastatin treatment. Pathological findings of glomerular sections that Ang II-induced PAS-positive deposition, TGF-β1 expression and oxidative stress evaluated by dihydroethidium staining were enhanced in eNOS KO mice, which were attenuated by pitavastatin treatment. Survival rate of eNOS KO mice was decreased by Ang II treatment; however, pitavastatin significantly improved the survival rate of Ang II-treated eNOS KO mice.

Androgen Increases Expression of Aortic At1a Receptors and Restores Angiotensin II-induced Abdominal Aortic Aneurysms in Castrated Male Apoe-/-- Mice

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Objective: Previous studies demonstrate that castration of male apoe-/-- mice reduces angiotensin II (Ang II)-induced abdominal aortic aneurysms (AAA) formation to the level observed in non-castrated (intact) male and female mice. Five weeks later, AT1a mRNA abundance was determined in the abdominal aorta. In intact male and female mice, AT1a mRNA abundance was greater in the abdominal than thoracic aorta. However, the fold difference in AT1a mRNA abundance between abdominal and thoracic aorta was greater in male (4.57-fold) compared to female (2.18-fold) mice. Castration of male mice reduced AT1a mRNA abundance by 61% in abdominal, but not thoracic aortas, resulting in similar AT1a mRNA abundance between these aortic regions. DHT administration partially restored AT1a mRNA abundance in castrated male mice (AT1a mRNA/18S RNA: intact, 1.74 ± 0.54, orchectomy/DHT, 1.14 ± 0.08). To determine if this dose of DHT would restore AAA susceptibility in castrated male mice, we infused saline or AngII to castrated male mice administered placebo or DHT. Administration of DHT to castrated male mice increased AAA incidence by 27% to 75%, but had no effect on AngII-induced athero-infarction. Conclusions: These results suggest that androgen positively regulates the expression of the AT1aR in abdominal aorta to increase AAA susceptibility in male apoe-/-- mice.

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Hyperbaric Oxygen Induces bFGF and HGF Expression and Enhances Blood Perfusion as well as Muscle Regeneration in Mouse Ischemic Hind Limbs

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Background: It is not clear how hyperbaric oxygen (HBO) affects ischemia-induced pathophysiological responses such as angiogenesis and skeletal muscle regeneration. We studied the effects of HBO on the functional and morphological recovery of ischemic hindlimbs, blood perfusion and the local production of angiogenic factors in a mouse model. Methods and results: Mice were placed in pure oxygen under 3 atm one hour a day for 14 days after the removal of a segment of left femoral artery. HBO-treated mice showed better functional recovery and greater blood flow in the ischemic hindlimb than untreated mice. Histological examination revealed the unatrophic muscle fibers with islands of small regenerating muscle cells and angiogenesis only in HBO-treated mice. Regeneration of muscle was confirmed by the increase in myf5 mRNA. The amount of mRNA for VEGF, HGF and bFGF was slightly increased in the ischemic hindlimbs. HBO eliminated the increase in VEGF mRNA. In contrast, the amount of mRNA for HGF and bFGF was significantly increased by HBO treatment. These results show that HBO stimulates the recovery of ischemic hindlimbs by increasing the production of bFGF and HGF and by promoting muscle regeneration in mice.

Statin Treatment of Vascular Endothelial Cells Disrupts Caveolae via Caveolin Depletion and Redistribution of Caveolin-1 Expression

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Statins inhibit a rate-limiting enzyme in the biosynthesis of cholesterol, 3-hydroxy-3-methylglutaryl-CoA reductase, and are widely used to treat atherosclerosis. In addition to its role in atherosclerotic plaque development, cholesterol is a requirement for the formation of caveolae. We have shown that palmitate induces a decrease in caveolae in vascular endothelial cells. In the current study tests the hypothesis that statin will have a direct effect on vascular endothelial cells, in particular affecting the relationship between cholesterol and caveolae abundance and localization. We studied human umbilical vein endothelial cells and bovine aortic endothelial cells with lovastatin or simvastatin treatments. Confocal imaging of caveolin-1 and eGFP and fluorescently-tagged cholesterol analogues in live cells identified a concentration- and time-dependent reduction of caveolin-1 and cholesterol at the plasma membrane in response to statin, respectively. Immuno-fluorescent experiments determined that statin reduced the caveolin-1 expression in buoyant sucrose-gradient fractions of Triton-X100-solubilized lysates without a significant change in total cell caveolin-1 expression. However, high concentrations (>1μM; >48hr) of statin did reduce the global caveolin-1 expression but this correlated with an increase in expression of the p17 fragment of caspase 3 indicating this may be a cytotoxic effect of statin at high doses. Since caveolae are a site of endothelial nitric oxide synthase (eNOS) and interaction with caveolin-1 has been shown to negatively regulate eNOS activity, we measured nitric oxide production (using an electrochemical sensor) in real-time from statin-treated cells, responses elicited by bradykinin (10nM) treatment of statin-treated cells were >2-fold those of control-treated cells. In conclusion, the results indicate that by acting directly on endothelial cells can produce a cascade of events initially reducing cholesterol content of plasma membrane; a subsequent reduction in caveolin-1 expression at the membrane; followed by an increase in eNOS activity. In addition to the cholesterol-lowering effects of statins these direct actions of statin on endothelial cells may underlie some of their therapeutic benefit in vivo.

Low Endothelial Shear Stress (ESS) Leads to Excessive Expansive Remodeling of Coronary Atherosclerotic Subsegments

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Background: The role of excessive expansive remodeling in the natural history of atherosclerosis has not been studied. We investigated the hypothesis that low ESS leads to the excessive expansive remodeling of coronary atherosclerotic subsegments. Methods: In 11 diabetic hyperlipidemic swine, IVUS based 3D reconstruction of the coronary arteries was performed at baseline (wk 23) and follow up (wk 36). Local ESS was assessed using computational fluid dynamics software and plaque-free subsegments (n=225) or IR (Fig a). Baseline and ESS were subcategorized into subsegments with EER or CER, and slightly increased in subsegments with IR (Fig b). Follow up ESS remained low in subsegments with EER or CER, and slightly increased in subsegments with IR (Fig b). Results: Subsegments with EER had larger plaques with more lipid deposition, inflammation and collagen content (p<0.05 or IR (Fig a). Baseline and ESS were subcategorized into subsegments with EER as compared to those with IR (Fig b). Follow up ESS remained low in subsegments with EER or CER, and slightly increased in subsegments with IR (Fig b). Conclusion: Low ESS leads to EER in atherosclerotic subsegments, and in this setting, the adverse low ESS environment persists, thereby fostering continued lipid accumulation, inflammation, matrix degradation, and development of a high risk plaque.
P208 Therapeutic Approach of Human Embryonic Stem Cell–Derived Endothelial Cells in Mouse Ischemic Heart and Hind Limb

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Human embryonic stem cells (HESCs) have been established as a potential resource for cell replacement therapy. Here, we determined the engraftment and reconstitution of functional endothelial-like cells differentiated from HESCs (HES-ECs) in NODSCID mice. HES-ECs were obtained from blast colonies and displayed endothelial characteristics by expression of KDR, VE-Cadherin, vWF, Tie-2, and uptake of LDL. To monitor engraftment of HES-ECs, HESCs were transfected with lentivirus-GFP/luciferase. HES-ECs (1×10⁵) were injected into either myocardial infarction (MI) mouse heart or ischemia hind limb (HL). Medium-only injections served as a control (n=6). Cell engraftment was determined at 2w and 4w (weeks) of cell transplantation in both MI hearts and HL by luciferase imaging. Doppler perfusion imaging was also performed to evaluate liver blood flow by comparison of the ratio of blood flow in HL to that in non-HL. Mice were sacrificed at 4w of cell transplantation to determine myocytes regeneration and new vessel formation derived from the HES-ECs in both MI hearts and IHL. A positive Luciferase image was obtained in both MI hearts and IHL at 2w and 4w of cell transplantation. Doppler imaging indicated that HES-EC transplantation significantly improved blood flow as early as 1w with a blood flow ratio (HL/non-HL): 0.6±0.2 in the HES-EC group versus the control group 0.3±0.1 (blood flow ratio at day 3 of ischemia ligation was 0.2±0.1). The blood flow in HES-EC mice was greatly improved thereafter in comparison to control mice. At 4w, cells positively stained with cardiac specific protein α-actin or Nkx2.5 were also positive for GFP in MI hearts, which indicated regeneration of myocytes from transplanted HES-ECs. New vessel formation was demonstrated by human specific vWF staining in both MI hearts and HL with vascular-like structure. Our data suggest that HESC-derived endothelial precursors could be used therapeutically for the treatment of ischemia heart and limb.

The Frequency of Cyclic Stretch Determines the Proliferative and Apoptotic Capacity of Vascular Smooth Muscle Cells in Vitro

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Cyclic stretch is a key factor in determining the proliferative and apoptotic capacity of vascular smooth muscle cells (SMC). Changes in SMC growth are critical to vascular remodeling and restenosis following injury. The aim of this work is to investigate the effect of cyclic stretch applied at different frequencies on the proliferation and apoptosis of SMC. Bovine aortic SMC were subjected to 5% cyclic stretch at different frequencies (0.5 and 1 Hz) for up to 96 h using a Flexercell Tension Plus FX-4000™ system with an applied equibiaxial heartbeat waveform before cell proliferation and apoptosis was evaluated. The Vybrant™ CDA-SE dye and Vybrant™ Alexa Fluor 488™ Annexin V and propidium iodide were utilized to determine cell proliferation and apoptosis, respectively, using FACS analysis. Cell counts were also performed using a hemocytometer to confirm changes in cell growth after a 24, 48 and 96 h period of the applied stretch. Using both FACS analysis and cell counting it was found that cyclic stretch decreased SMC proliferation in a temporal manner. Moreover, when cells were exposed to the lower frequency, SMC proliferation was inhibited to a greater extent. (Fig 1). In parallel cultures using FACS analysis, the level of SMC apoptosis following exposure to cyclic stretch was also frequency dependent. There was a reduced level of apoptosis at 96 h at the higher frequency. We conclude that cyclic stretch has a temporal antiapoptotic effect on SMC in vitro. When cells are subjected close to the physiological frequency condition, the growth and apoptotic activity of these cells is reduced.

A Role for Formin Homology Proteins in Shear Stress–induced Endothelial Cytoskeleton Remodeling

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The frictional drag caused by the laminar flow of fluid over the surface of cells causes a shear stress to the underlying cell layer. Endothelial cells shifted from static to flow culture conditions undergo a unique and stereotypical response to this stimulus. They respond as a population to re-pattern their cell–cell junctions and cytoskeletal networks to change from a ‘cobblestone’ appearance to a more elongated morphology that is polarized in the direction of flow. This shear–stress induced morphology is also observed in vivo and is thought to be atheroprotective. Formins are cytoskeleton remodeling proteins that regulate polymerization of actin filaments. The cytoskeletal structures that are induced by activation of formin proteins are highly homologous to the structures that are formed in endothelial cells in response to shear stress. The function of formin proteins in endothelial cells in any context has not been addressed. Therefore we wished to test the hypothesis that Formin proteins are required for endothelial cytoskeleton remodeling in response to flow–induced shear stress. Using RT–PCR we have shown that the five same name formin family members are expressed in endothelial cells. To examine the expression of the nine formins in endothelial cells, we transfected with lentivirus-GFP/luciferase. HES-ECs (3×10⁵) were injected into either myocar-

The Effect of Rosiglitazone and Pioglitazone on Myocardial Apoptosis

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Background: The role of apoptosis in heart failure and repair/fusion injury has been established by previous studies. PPAR-gamma agonists rosiglitazone and pioglitazone are widely used for the treatment of diabetes mellitus and insulin resistance and also have favorable effects on lipid profile. Glitazones, on the other hand, are associated with worsening of heart failure. Thus, we aimed to investigate the effect of glitazones on myocardial apoptosis.

Methods: H9c2 rat myocardial cell cultures were used as a model of apoptosis. Doxorubicin was used to induce apoptosis. MTT assay was performed to measure cellular cytotoxicity. Reactive oxygen species (ROS) was taken as a surrogate of cytotoxicity. Intracellular ROS measurements were assessed by a fluorescent reader using 5,6-Chloromethyl-2,7-dichlorodihydrofluorescein diacetate-acetyl ester (CM-H2DCFDA) in living cells. Caspase-3 and caspase-9 levels were determined to assess myocardial apoptosis.

Results and Conclusion: Although glitazones are clinically associated with aggravation of heart failure, the addition of rosiglitazone (10 μM) resulted in attenuation of cellular toxicity caused by doxorubicin in cardiomyocytes. Additionally, rosiglitazone treatment decreased doxorubicin-induced ROS generation significantly (p<0.01) in living myocardial cells. Doxorubicin treated cells displayed higher caspase-3 and caspase-9 levels compared with controls, which are markers associated with apoptosis. Rosiglitazone attenuated the elevation of both caspase activities at significant levels (p<0.05). Rosiglitazone decreased doxorubicin-induced caspase 3 activity by nearly a half from 231.98±9.01% (caspase activity) to 127.71±15.91. Caspase 9 activity went down from 225.74±15.38 to 172.37±2.75. These findings were comparable to those of the well known antioxidant, N-acetyl L-cystein. We also found similar antioxidative and anti-apoptotic effects for pioglitazone (data not shown). As a conclusion, neither rosiglitazone nor pioglitazone attenuate cellular cytotoxicity and apoptosis in doxorubicin treated myocardial cell cultures in an in vitro setting, although glitazones are known to be associated with aggravation of heart failure.

PTEN Deletion Enhances Vascular Smooth Muscle Cell Proliferation and Accelerates Neointima Formation

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A change in proliferative phenotype of vascular smooth muscle cells (SMC) from a highly quiescent to a rapidly proliferating phenotype is a key component of neointima formation. The PI3K/Akt pathway is a principal pathway involved in SMC proliferation and phenotypic modulation. PTEN is a tumor suppressor gene that negatively regulates the PI3K pathway; our previous research has shown that early inactivation of PTEN in response to vascular injury is a critical event involved in neointima formation. The goal of this study was to determine the role of PTEN in SMC function and neointima formation using both in vitro and in vivo techniques. Rat aortic SMC were transfected with control or PTEN-specific siRNA oligonucleotides. Western blotting was used to determine levels of total PTEN and phospho-Akt and SMC proliferation was determined by BrdU immunohistochemistry. To study the effect of PTEN depletion in vivo, we performed carotid artery ligation experiments on wild type mice (WT), global PTEN heterozygous mice (−/−) and caspase-15 Cre;PTEN −/− mice. At day 13 following injury, mice were injected with BrdU and tissues harvested on day 14. Uninjured and injured carotid arterial segments were examined for neointima size and were stained for SM-α-actin and BrDU using immunofluorescence/histochemistry. Compared to controls, SMCs transfected with PTEN siRNA exhibited significantly decreased PTEN levels with corresponding increases in phospho-Akt and enhanced proliferation under both basal and serum-stimulated conditions. Injured carotid artery ligation experiments further showed that global PTEN deletion significantly increased neointima size and reduced SMC proliferation.
Ca²⁺ is a ubiquitous second messenger and controls many cellular processes in vascular smooth muscle, including proliferation. Activating Ca²⁺-permeable channels of the transient receptor potential (TRP) family contributes to smooth muscle proliferation. Specific TRP channel isoforms can be regulated by the sarcoplasmic reticulum (SR) Ca²⁺ stores, such that depolarizing the SR of Ca²⁺-coupled to influx of Ca²⁺. We tested the hypothesis that Osabaw pigs that manifest the metabolic syndrome when fed excess fat/cholesterol diet have greater TRP channel-mediated Ca²⁺ influx compared to WT mice. Immunohistochemistry showed that the neointimas were strongly Ca²⁺/H11005-permeable TRP channels may contribute to smooth muscle cell proliferation.

Hypercholesterolemia in the Metabolic Syndrome Increases the Functional Expression of TRP Channels in Coronary Myocytes from Osabaw Miniature Swine

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cardiovascular disease (CVD) is a major cause of mortality and morbidity in human beings. The burden of CVD increases with age, and the risk factors for CVD are often chronic and may be modulated by lifestyle. In the metabolic syndrome (MetS), insulin resistance (IR) and obesity are associated with CVD and hypertension. The MetS is considered to represent a proatherogenic state and increased risk of diabetes and CVD. However, it remains controversial whether the MetS is a main risk factor or modulator of arterial wall remodeling.

4h, 1d, 2d after surgery and at discharge. Peripheral blood leukocytes (PBL) from healthy donors were isolated (Ficoll-Hypaque centrifugation) and 250,000 cells were placed into a migration chamber (Costar) separated from a second lower chamber filled with patient serum (20% in RPMI) by a filter (pore size 3μm). After incubation (1h, 5% CO₂, 37°C) cells from top and bottom chamber were removed and analysed by flow cytometry. Results: Chemotactic activity increased at onset of anaesthesia followed by a phase of low activity immediately after surgery and a second phase of high activity at post-operative days 1-2. In the first phase mainly monocytes and NK-cells migrated. The in vitro results correlated with results obtained by immunopenotyping of circulating PBL of the same patients showing that at CPB onset monocyte and NK-cell count increases. After surgery of T- and B-cell count decreased probably due to homing into lymphatic tissues. From both chambers the total number of cells recovered was 5-15% below that of the initial cell number due to attachment of migrating cells to the filter, which was quantified by LSC.

Discussion: During paediatric cardiac surgery the chemotactic activity of the serum changes following characteristic patterns. Manipulation of the chemokine pattern might prove beneficial to prevent extravasation of cells leading to tissue damage.

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Progenitor Cells: A Role in Vascular Calcification?

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Vascular calcification is an important determinant of cardiovascular mortality. It has been suggested that bone marrow derived progenitor cells may contribute to the development of arterial calcification. In this study we investigated the contribution of the circulating progenitor cells towards the development of vascular calcification using an Osteoprotegerin knockout (OPG−/−) murine model system, previously shown to develop calcification. The degree of vascular calcification in the aorta was evaluated using an alginate cast and a bioassay system for the quantification of calcium (∼10−16). Tail bloods (n=104) regular intervals were analysed for progenitor cells such as stem cell antigen (Sca-1) and c-kit (CD117) using flow cytometry. The mean degree of calcification was observed to be more in experimental (OPG−/−) mice (0.45±0.03 mm) than the control (C57BL/6) mice (0.21±0.02 mm). Comparative studies also showed that the percentage population of progenitor cells were significantly lower in the OPG−/− mice (P<0.05) in the OPG−/− as compared to the C57/BL6 mice (Table 1). The degree of calcification was correlated to the percentage population of the progenitor cells. Bone markers such as osteopontin (P<0.001), osteoclast (P<0.001) and bone alkaline phosphatase (P<0.01) were also expressed in Sca-1, c-kit positive cells. In conclusion, our findings suggest that circulating progenitor cells may contribute to the development of arterial calcification. Our findings have implications for bone marrow based cellular therapies being developed.

TABLE 1: C57 VS OPG−/−: COMPARISON OF PERCENTAGES OF PROGENITOR CELL POPULATION

Mice (n=104) Tail bloods (n=104) Sca +/c-kit- Sca +/c-kit + OPG−/− (experimental) 0.78(2.09) 0.28(0.05) 0.19(0.02) C57 (control) 1.47(0.11) 0.68(0.06) 0.43(0.05)

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Differential Contribution of Cyclooxygenase-Isozymes to the Generation of Prostacyclin and Prostaglandin E₂ by Endothelial Cells in Response to Steady Laminar Shear Stress

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Prostacyclin (PGI₂) and prostaglandin(PGE₂), the major prostanoids released from endothelial cells, play different roles in cardiovascular(DV) homeostasis. PGI₂ is a general restraint on endogenous stimuli to platelet activation, vascular proliferation and remodeling, atherogenesis, and cardiac function. Differently, PGE₂ accelerates atherogenesis and can activate platelets. We explored the contribution of cyclooxygenase (COX)-isozymes and down-stream specific syntheses to the generation of PGI₂ and PGE₂ in endothelial cells in response to steady laminar shear stress (SSS). Serum samples were obtained 1d preoperative, after anesthesia, at the onset and the end of the CPB,

The Chemotactic Potential of Serum of Patients Undergoing Cardiac Surgery: The Time Course of Chemotactic Potential from Different Times of Surgery

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Cardiac surgery with cardiopulmonary bypass (CPB) induces massive perturbations of the immune system. Leukocyte composition in the peripheral blood. This immune response contributes to the adverse outcome with migration of activated cells to sites of inflammation that is driven by attractant and repellent chemokines acting in concert. Patients and methods: Nine patients undergoing cardiac surgery with CPB were studied. Serum samples were obtained 1d preoperative, after anesthesia, at the onset and the end of the CPB,
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Activin A and Transforming Growth Factor-β1 Have Distinct Functions in Smooth Muscle Cell Migration
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Activin A and Transforming Growth Factor-β (TGF-β) are members of the TGF-β superfamily. Both modulate vascular lesion formation beneficially, however, they involve distinct mechanisms in this process. Activin A inhibits the formation of smooth muscle cell (SMC)-rich lesions, whereas TGF-β1 does not. In addition, TGF-β1 induces production of extracellular matrix components, resulting in a larger but more stable vascular lesion. Activin A and TGF-β1 bind unique cell-surface receptors, but both involve Smad-2, 3 and 4 in downstream signaling pathways. To understand how, Pin and Activin A after SMC-rich lesion formation differently, we first investigated the presence of Activin receptor-like kinases (ALK) in SMCs. We demonstrate that ALK-1, 2, 4, and 5 are all expressed in human SMCs. Western-blot analyses showed that both Activin A and TGF-β1 inhibit Smad-2 phosphorylation, however, also Smad-1 phosphorylation was observed. This is contrary to our knowledge so far. Furthermore, we have not been reported before to phosphorylate Smad-1, this was unexpected. Secondly, we performed micro-array experiments followed by gene-set enrichment analyses on SMCs stimulated with either Activin A or TGF-β1. Several pathways were discovered to be differentially regulated by these factors, including cellular migration. Furthermore, we confirmed that Activin A does not affect SMC migration (323.6 ± 22.7 mm² vs. control: 330.1 ± 17.2 μm², after 24 hours; n=8, p>0.05), whereas TGF-β1 inhibits migration (249.9 ± 17.1 μm² vs. control: 330.1 ± 17.2 μm², after 24 hours; n=6, p<0.01), most likely through increased expression of extracellular matrix proteins promoting firm attachment of the cells. In conclusion, gene expression profiling experiments provide evidence that Activin A and TGF-β1, in spite of the activation of intracellular signaling pathways, provoke significant differences in cellular responses of SMCs, which may clarify their distinct effects on the vessel wall.

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Calcium/CaMulin-Dependent Protein Kinase II-δ Regulates Vascular Smooth Muscle Cell Proliferation
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There is accumulating evidence that Ca2+ -dependent signaling pathways regulate proliferation and migration of SMC cells contributing to the intimal accumulation of SMC observed in many vascular diseases. In this study we investigated the role of the multifunctional serine/threonine kinase, CaMIIIδ, as a mediator of Ca2+ signals regulating VSMC cell proliferation. Differentiated VSM cells isolated from rat aortic media express preferentially CaMIIδ gene products while passed primary cultures of de-differentiated VSM cells express primarily CaMIIδ. Experiments examining the time course of CaMIIδ isoform modulation revealed the process was rapid following initial dispersion of aortic VSM with a significant increase in CaMIIδ protein and significant decrease in CaMIIγ protein within 30 hrs, coinciding with onset of DNA synthesis and cell proliferation. Attenuating the early upregulation of CaMIIδ, in primary cultured cells using siRNA resulted in decreased serum-stimulated DNA synthesis and cell proliferation. In passaged VSM cells, suppression of CaMIIδ activity by overexpression of a kinase-negative mutant, or suppression of endogenous CaMII content using multiple siRNAs, confirmed that Activin A does not affect SMC migration (323.6 ± 22.7 mm² vs. control: 330.1 ± 17.2 μm², after 24 hours; n=8, p>0.05), whereas TGF-β1 inhibits migration (249.9 ± 17.1 μm² vs. control: 330.1 ± 17.2 μm², after 24 hours; n=6, p<0.01), most likely through increased expression of extracellular matrix proteins promoting firm attachment of the cells. In conclusion, gene expression profiling experiments provide evidence that Activin A and TGF-β1, in spite of the activation of intracellular signaling pathways, provoke significant differences in cellular responses of SMCs, which may clarify their distinct effects on the vessel wall.

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Alovastatin Attenuates Lp-r-mediated Aortic Valve Calcification and Osteoporosis in LDLr-/- Hypercholesterolemic Mice
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Cardiovascular calcification and osteoporosis are the leading causes of morbidity and mortality in the aging population in the United States. Evidence suggests that aortic valve calcification and osteoporosis are related and share common pathogenesis. We have previously shown that aortic valve calcification expresses an osteoblast phenotype in humans (Rajamannan et al, Circulation. 2003 May 6;107(17):2181–8). We hypothesize that C3H10T1/2 cells, a multipotent mesenchymal stem cell line, can differentiate into bone-forming cells in the presence of aortic valve c-Myc transcription, protein kinase A and Rho. In vivo model of CV after SAH uses CSF from SAH patients with CSF and without CSF. We compared the effects with these laboratory-attenuated bovine aortic valve carotid arteries (PCA) and examined the activation of regulatory proteins PKCα, PKCδ and Rho. PKCα, PKCδ, and Rho are pre-stretched to spay tension and placed in control solution (MOPS), CSF, CSF and BOXes (25 μM) for 2 hours. After 3 hours and translocation of PKCα, δ and Rho from cytosol to membrane is analyzed using western blot. Among different protein kinase C isoforms, α and δ are present in appreciable amounts in porcine carotid artery smooth muscle. Translocation of PKCα, δ and Rho from the cytosol to the membrane indicates activation of PKCα, δ and Rho in the presence of CSF, and BOXes when compared with CSF and BOXes respectively. The temporal changes in translocation of PKC-α, δ and Rho are likely to be important for the initiation and maintenance phases of valve calcification and will require further study to determine their relative contributions to pathology. BOXes are likely the major vasoactive compounds in CSF, that causes the activation of these contractile proteins and results in increased calcium sensitization of the vascular smooth muscles.

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Wnt3a Upregulates SM22α Gene Expression in C3H10T1/2 Mesenchymal Cells via a Novel CAGAG Regulatory Element
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The regulation of β-catenin-dependent gene transcription has been implicated in the proliferation and phenotypic modulation of vascular smooth muscle cells (VSMC) during both development and disease. In order to better understand the role of Wnt-dependent control of the VSMC phenotype, we examined Wnta’s effects on C3H10T1/2 (10T1/2) cells, a multipotent mesenchymal progenitor capable of adopting the mural VSMC phenotype in vivo. Following Wnta treatment, 10T1/2 cells developed a spindle-shaped, myofibroblast phenotype in culture. Treatment with 5 ng/ml to 3 ng/ml of Wnta in 2% horse serum significantly upregulated the expression of early VSMC markers such as SM22α (5-fold) and smooth muscle α-actin (2-fold).
Cyclical Strain of Vascular Endothelial Cells Reduces Vascular Smooth Muscle Cell Proliferation, Possibly via an MMP-2-dependent Mechanism

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Introduction: Hemodynamic forces, namely shear stress and cyclic strain play an important role in physiological control of vascular tone, remodelling and associated pathologies. Furthermore, these forces indirectly impact vascular smooth muscle cell fate decisions by modulating vascular endothelial growth factor (VEGF) expression and SM22α messenger RNA (mRNA) levels in vascular cells in culture via degradation of extracellular matrix substrates. Recent studies demonstrate that these forces can modulate MMP expression and activity in different vascular cell types. For these investigations, we examined how MMP-2 and MMP-9, induced in BAECs in response to cyclical strain, putatively impact on BASMC proliferation. Methods: BAECs were exposed to a defined level of equibiaxial cyclic strain using the Flexercell® Tension Plus ™ FX4000™ apparatus (10% strain, 60 cycles/min, 24 h, cardiac waveform). BAECs were harvested for Real Time PCR to monitor MMP-2 mRNA levels whilst BASMCs were incubated under similar conditions. BASMCs were then stimulated with incremental concentrations of cyclical strain (50%, 100% and 150%) and harvested for FACS analysis and cell counting. Results: Cyclic strain of BAECs increased MMP-2 and MMP-9 mRNA levels by (1.2±0.1 and 1.9±0.1 fold, respectively). Subsequent incubation of BAECs with BOC decreased proliferation to (0.86±0.1 and 0.89±0.1 fold, respectively) relative to control cells. These findings indicate that cyclical strain of BAECs reduces BASMC proliferation. Moreover blockade of MMP-2 induction but not MMP-9 appeared to reverse this effect, suggesting a regulatory role for strain-induced endothelial-derived MMP-2.

Three-day High-flow Loaded Rabbit Carotid Artery Remodels Automatically Ex Vivo

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[Introduction] High blood flow induces endothelial cell proliferation and arterial dilation. Arterial dilation starts as the tearing of internal elastic lamina (IEL gaps) at 4-day high flow in the rabbit carotid arteries. As IEL gaps widen, arteries dilate. At first we believed that high flow was necessary during whole process. However, we wonder whether high-flow is necessary in the whole process or transient high-flow is sufficient for the remodeling. Here, we loaded 3-day high-flow to the rabbit carotid artery and then removed the artery for ex vivo to observe whether internal elastic lamina might be torn without flow.Method/In order to increase blood-flow, arterio-venous fistula (AVF) was created in the left common carotid artery and jugular vein. 3 days after creation of AVF, left common carotid artery was removed and the whole blood vessel was cultured in vitro (ex vivo) for 1 day. After 1-day, carotid arteries were observed with light microscopy and laser scanning microscopy (LSM) (n~6). Non-operated animals served as controls. Results/As compared with controls, subendothelial thickness was decreased in the ex vivo cultured artery. They were small and occasional. No IEL gaps were observed in the controls. MIB-5 positive smooth muscle cells were observed in the media beneath IEL gaps. [Discussion] We proved that 3-day high-flow loaded rabbit carotid artery gaps increased blood flow, arterio-venous fistula (AVF). Therefore it is proposed that 3-high-flow loaded rabbit carotid artery remodeling automatically ex vivo. We assume that the cause of remodeling has already played and arranged within 3-day of high-flow, although no detectable morphological initiation could be detected. We suspect that transient high-flow may incuate smooth muscle progenitors into the arterial wall.

High-density Lipoprotein Cholesterol Prevents Apoptosis in Endothelial Progenitor Cells Through Activation of AMP-activated Protein Kinase

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Endothelial dysfunction is thought to play a critical role in the atherosclerosis. Emerging evidence suggest pluripotent cells present in the peripheral blood called endothelial progenitor cells (EPCs) help in maintaining the endothelial integrity during atherosclerotic vascular disease. Therefore enhancing the number and function of EPCs should be beneficial in preventing vascular disease. High-density lipoprotein (HDL) cholesterol has shown promising results in protecting endothelial cell monolayer and reverse endothelial dysfunction. HDL might exert at least in part its beneficial effect on vascular endothelium through EPCs mobilization. However, HDL is known to exert extra cellular regulatory effects and in the beneficial effect of HDL is not completely understood. We hypothesized that AMP-activated protein kinase (AMPK), an intracellular kinase involved in cellular energy regulation, may constitute a key signalling enzyme in the beneficial effect of HDL on EPCs. Methods and Results: We isolated EPCs from peripheral blood in healthy subject using standard procedure. Apoptosis was induced in EPCs by camptothecin. To assess protective effect of HDL on EPCs, we pre-treated EPCs with either HDL or phenoformin. Apoptotic cell population was quantified by flow cytometry. We found that HDL reduced apoptosis in EPCs (HDL+ Camptothecin 19.6±0.6% vs. Camptothecin 24.4±1.4% p<0.0001). Furthermore, we showed that an AMPK inhibitor (phenformin) only slightly reduced the apoptosis of EPCs (Phenformin + Camptothecin 17%±0.6 vs Camptothecin 24.4%. p=0.03±0.0001). Finally, we determined that HDL-induced protective effect on EPCs is mediated through the activation of AMPK, which was measured by Western Blot and densitometry. Conclusions: Our results indicate that HDL has potent anti-apoptotic effects on EPCs, which might be a contributory factor towards its anti-atherosclerotic action. We found that HDL can reduce camptothecin-induced apoptosis in EPCs. Furthermore, we also found that this beneficial effect of HDL is mediated via activation of AMPK. Interestingly, activation of AMPK by anti-diabetic drug phenformin also produces similar beneficial effects in EPC. Therefore our study underlines a promising role of AMPK stimulation in endothelial homeostasis.
Synergistic Interaction Between Aldosterone and Angiotensin II: Differential Effects on RhoA, Akt, and MAP Kinase Signaling in Vascular Smooth Muscle Cells

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Aldosterone (Aldo) exerts a synergistic mitogenic effect with angiotensin II (Ang II) through ERK and JNK activation in vascular smooth muscle cells (VSMC). Whether similar interactions influence contractile and migratory signaling pathways remain unclear. Here we investigated cross-talk of c-Src, MAP kinases (ERK 5, p38 and JNK), RhoA, Akt and [Ca2+]i signaling between Aldo and Ang II. Cultured rat mesenteric vascular smooth muscle cells were studied. Activation of MAP kinases and Akt was determined by immunoblotting using phospho-specific antibodies. Rho activity was assessed by the G-LISA Rho activation assay kit. [Ca2+]i was measured by a fura-2 methodology and fluorescence digital imaging. Whereas high concentrations (~10-5 mol/L) of Aldo and Ang II significantly increased activation of c-Src (~2-fold), low concentrations (~10-8 mol/L) of both had no effect. Concentration-dependent effect. Co-stimulation of VSMCs with combined low dose Aldo and Ang II significantly increased c-Srphorylation (0.5-fold above basal, p<0.05), Rho activity (1-fold, p<0.05) and [Ca2+]i transients (~1-2-fold) and decreased Akt phosphorylation (0.5 fold, p<0.05). ERK 5, p38 and JNK were unaffected by low dose Aldo/Ang stimulation. Synergistic effects were selective for reconstituted in vitro (RAT) kinase, i.e., i/n esterase, i/n scarocoid receptor (MP) antagonist, euglenore (10-6 mol/L), or a mineralocorticoid receptor (MR) antagonist, euglenore (10-6 mol/L). Our results suggest that Aldo and Ang II act in a synergistic fashion to regulate defined signaling pathways, particularly those related to VSMC migration and cell survival.

Air Pollutant Chemicals and Oxidized Lipids Exhibit Genome-wide Synergistic Effects on Endothelial Cells

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Background - Ambient air pollution is associated with increased cardiovascular morbidity and mortality. We have recently found that exposure to ambient ultrafine particulate matter, highly enriched in redox cycling organic chemicals, promotes atherosclerosis in mice. We hypothesize that these pro-oxidative chemicals could synergize with oxidized lipid components generated in low-density lipoprotein (LDL) particles to enhance vascular inflammation and atherosclerosis. Methods and Results - We have used human microvascular endothelial cells (HMEC) to study the combined effects of a model air pollutant, diesel exhaust particles (DEP), and oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (ox-PAPC) on genome-wide gene expression. We treated HMEC in triplicate wells with an organic DEP extract (5 μg/ml, ox-PAPC (10, 20 and 40 μg/ml) or combination of both compounds for 4 hours. Gene expression profiles were assessed by Illumina microarray technology and validated by qPCR. Both the DEP extract and ox-PAPC co-regulated a large number of genes. Altogether, 1555 genes were significantly upregulated (~1.5 fold, p<0.05) by the three DEP and ox-PAPC combinatory treatments. We used weighted gene co-expression network analysis on the 3600 most varying genes to identify co-expressed gene modules. We found three modules that were most highly enriched in genes differentially regulated by the stimuli. These modules exhibited a pattern of additive/synergistic interaction and concentrated 83% of the genes that were synergistically upregulated by both DEP and ox-PAPC. These modules were also enriched in pathways relevant to vascular inflammation such as antioxidant, apoptotic, inflammatory and unfolded protein response. We validated this synergic in vivo by demonstrating that liver gene expression of apoe null mice exposed to ambient ultrafine particles and fed a high fat diet exhibited significantly upregulated expression of the module genes. Conclusions - Diesel exhaust particles and oxidized phospholipids synergistically affect the expression profile of several gene modules, that correspond to pathways relevant to vascular inflammatory processes such as atherosclerosis.

Synergistic Effects of Diesel Exhaust on Endothelial Cells

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Diesel particulate instillation dysregulates the endothelial transcriptome

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Air pollution accelerates atherosclerosis and cardiovascular disease. We examined the effect of diesel exhaust on endothelial gene expression in vivo, utilizing hypercholesterolemic transgenic mice. Tie2-GFP mice expressing green fluorescent protein regulated by the endothelial-specific promoter Tie2 were crossed with apolipoprotein E knockout mice to generate Tie2-GFP/ApoE null mice. Tie2-GFP and Tie2-GFP/ApoE mice were exposed to NIST diesel exhaust particulate by intratracheal instillation of 100 μg. Within 24 hours of exposure, aorta (poled from 4 animals) were dissected from the valve to the iliac bifurcation and rapidly processed by proteolytic dissociation followed by fluorescence activated cell sorting to yield > 100000 endothelial cells of > 95% purity. Changes in the abundance of endothelial transcripts were then examined comprehensively by microarray techniques. Amplified RNA representing > 25000 cells was subjected to fluorescent labeling and hybridization to arrays created from the OpenAverage Express 4 olio set. Within 1 day of DE exposure with a return to FA exposed levels with longer DE exposures. Interleukin-6 (IL-6), IL-1, IL-1β, IL-12, IFNγ and TNFα were not detected in lung lavage fluid at any time point. While IL-2, IL-4 and GM-CSF levels were unaltered, exposure to DE suppressed pulmonary IL-10 production. In plasma, there was no change in IL-2, IL-10, IL-12 or GM-CSF levels and IL-5 was not detected following DE exposure. In contrast, exposure to DE increased plasma levels of IL-1α, IL-4 and TNFα. Plasma levels of IL-1α, IL-4 and TNFα also increased and the increases demonstrate significant responses to DE. Based on the cytokines modulated, there appears to be both a cell mediated and antibody mediated response to DE. This change in circulating cytokines may directly affect the vasculature, cardiac muscle or atherosclerosis with potential to indicate adverse effects. The consistent upregulation in circulating cytokines that characterizes DE exposure is mediated by cytokines.

Glutathione Biosynthesis Genes Protect Macrophages from Diesel Exhaust–Mediated Toxicity

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There are a growing number of epidemiological studies linking cardiopulmonary morbidity and mortality to exposure to particulate matter, a major component of air pollution. These events are likely mediated by several mechanisms, including inflammation and oxidative stress. Oxidative stress can be attenuated by many antioxidants, including the tripeptide thiol glutathione (GSH). GSH biosynthesis is rate-limited by glutamate-cysteine ligase (GCL), which consists of catalytic (GCLC) and modiﬁer (GCLM) subunits. Macrophages play a dominant role in the pulmonary immune response to particulate matter and are also involved in the development of atherosclerotic plaques. Therefore, in order to study the protective role of GSH in the development of oxidative stress-related diseases, our laboratory utilized a RAW264.7 macrophage cell model in which GCLC and/or GCLM are overexpressed. Cells were exposed to 0, 50 or 200 μg/ml of diesel exhaust particles (DEP) for 18 hours and viability and GSH levels were measured using flow cytometry. There was a 2-3 fold increase in GSH levels in GCLC over expressing RAW cells. Exposure to 50 μg/ml DEP decreased survival of normal macrophages by 75%. Elevated GCLC expression rescued cells from DEP-mediated death, evidenced by a 2-fold increase in survival, while over expression of GCLM was not protective. These preliminary data demonstrate the importance of GSH and GCL in protecting macrophages from DEP-mediated toxicity.

Oxidative Stress Modification of Apolipoprotein E in Environmental Tobacco Smoke

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ApoB-E (apoE), an anti-atherogenic protein, plays a crucial role in cardiovascular diseases by regulating plasma cholesterol and lipoprotein metabolism. By assisting in the transportation of very low-density lipoproteins and high density lipoproteins, apoE is able to remove excess amounts of cholesterol and triglyceride from the blood and into the liver by transporting of very low-density lipoproteins and high density lipoproteins, apoE is able to remove excess amounts of cholesterol and triglyceride from the blood and into the liver by assisting in the development of oxidative stress-related diseases, our laboratory utilized a RAW264.7 macrophage cell model in which GCLC and/or GCLM are overexpressed. Cells were exposed to different levels of cigarette smoke and then examined comprehensively by microarray techniques. Amplified RNA representing > 25000 cells was subjected to fluorescent labeling and hybridization to arrays created from the OpenAverage Express 4 olio set. Within 1 day of DE exposure with a return to FA exposed levels with longer DE exposures. Interleukin-6 (IL-6), IL-1, IL-1β, IL-12, IFNγ and TNFα were not detected in lung lavage fluid at any time point. While IL-2, IL-4 and GM-CSF levels were unaltered, exposure to DE suppressed pulmonary IL-10 production. In plasma, there was no change in IL-2, IL-10, IL-12 or GM-CSF levels and IL-5 was not detected following DE exposure. In contrast, exposure to DE increased plasma levels of IL-1α, IL-4 and TNFα. Plasma levels of IL-1α, IL-4 and TNFα also increased and the increases demonstrate significant responses to DE. Based on the cytokines modulated, there appears to be both a cell mediated and antibody mediated response to DE. This change in circulating cytokines may directly affect the vasculature, cardiac muscle or atherosclerosis with potential to indicate adverse effects. The consistent upregulation in circulating cytokines that characterizes DE exposure is mediated by cytokines.
Pulmonary Diesel Pollution Exposure Dysregulates Key Cardiac and Pulmonary Transcripts

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Air pollution accelerates atherosclerosis and cardiovascular disease. We examined the effect of diesel exhaust particles on cardiac and pulmonary gene expression in vivo, utilizing transgenic mice. Tie2-GFP mice express green fluorescent protein regulated by the endothelial-specific promoter Tie2. Tie2-GFP mice were subjected to intratracheal instillation of a single dose of 100 μg of diesel exhaust particles. Within 1 and 5 days after exposure, the lungs (poled from 4 animals) were dissected and the total RNA was extracted. Changes in abundance of transcripts were then examined comprehensively by microarray techniques. RNA was subjected to amplification prior to labeling and hybridization to arrays created from the Operon V3 long oligo set. Within both 1 and 5 days after exposure to diesel exhaust particles, a subset of 11 transcripts detected by microarray were consistently dysregulated by more than 2 fold within heart. Within 5 days after exposure of diesel exhaust particles, an overlapping subset of 18 transcripts was consistently dysregulated by greater than 2 fold within both heart and liver. Within the heart of Angiotensin II-induced AAA mice (females, 21 days, 28 mmHg), 22 dysregulated transcripts were found. The expression of AChE and cathepsin D (known to have a low density lipoprotein receptor family of proteins and mediate effective clearance of plasma triglyceride-rich lipoprotein particles) has this potential implications in predisposing second-hand smokers to developing a pro-atherogenic profile and cardiovascular disease.

The Environmental Pollutant, Polychlorinated Biphenyl 77, Augments Angiotensin II-induced Abdominal Aortic Aneurysm Formation in Apolipoprotein E-deficient Mice

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Objectives: Polychlorinated biphenyls (PCBs) are omnipresent industrial pollutants that have been linked to increased cardiovascular disease. Previous studies demonstrated that co-planar PCBs (PCB77) increase proinflammatory gene expression in endothelial cells, macrophages and adipocytes. Intusion of angiotensin II (AngII) to hypertensive mice results in the formation of abdominal aortic aneurysms (AAA). AngII-induced AAs are associated with pronounced inflammation. The purpose of this study was to determine if PCB77 would augment AngII-induced AAs. Methods and results: Male ApoE−/− mice (3 months of age) were injected twice (6p) with either vehicle (safflower oil) or PCB77 (170 μM/kg) 1 week prior to and 1 week after implantation of omniporous minipumps for infusion of saline or AngII (1,000 ng/kg/min) for 28 days. Administration of PCB77 increased body weight gain in saline- and AngII-infused mice (vehicle, 1.4 ± 0.3; saline/PCB77, 3.6 ± 0.3; AngII/PCB77, 2.2 ± 0.4 and P = 0.0036). Elevations in body weight were associated with adipocyte hypertrophy in mesenteric adipose tissue and liver steatosis from PCB77-infected mice. PCB77 increased systolic blood pressure in saline-infused mice (vehicle, 102 ± 3 mmHg; PCB77, 117 ± 4 mmHg). Infusion of AngII increased blood pressure to a similar extent in vehicle (146 ± 6 mmHg) and PCB77-infected mice (140 ± 4 mmHg). Both AAA incidence (AngII/vehicle, 45%; AngII/PCB77, 90%) and aortic diameter measured by ultrasound (day 28: vehicle, 0.92 ± 0.01; AngII/vehicle, 1.61 ± 0.21; AngII/PCB77, 2.32 ± 0.15 mm, P < 0.05) were increased by PCB77. PCB77 also increased the severity of AAs, including mortality from ruptured AAs. Unexpectedly, PCB77 resulted in striking ectopic lipid deposition manifested in skeletal muscle (diaphragm), visceral peritoneum and liver. Conclusions: PCB77 promoted AngII-induced AAA formation and severity. Elevations in body weight, body fat and adiposity, and enhanced inflammation may have contributed to detrimental effects of PCB77.

Diesel Exhaust Enhances Vascular Oxidative Stress, Vasoconstriction, and Venous Congestion in a Cardiomyopathic Hamster Model

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Environmental air pollution has been associated with increased hospital admissions and death due to heart failure. However, the exact mechanism(s) by which environmental air pollution affects heart and vasculature is currently unknown. Recent studies have found that exposure to environmental air pollution enhances vasoconstriction in humans. We hypothesized that diesel exhaust (DE), a major component of ambient urban air, could enhance vasoconstriction and produce venous congestion in the presence of a failing heart. To test this hypothesis, we exposed older cardiomyopathic hamsters (HCM; fractional shortening 0.15–0.20, normal 0.60) to freshly derived DE. HCM were exposed to 300μg/m3 of DE for 4 hr/day for 2 days and venous pressures (Pven) were obtained via radio telemetry. Pven was significantly increased to ~13 mmHg compared to control animals (Pven ~5 mmHg). This was not increased in early-infarct hearts (based on histological analyses that pre-existing cardiac disease is requisite for the development of venous congestion). DE also enhanced vascular oxidant generation in exposed animals as measured by lucigenin-enhanced chemiluminescence. Interestingly, HCM animals exposed to gasoline exhaust at 60μg/m3, a 1:12 dilution with filtered-air, for 3 hr for 1 day did not have enhanced oxidant generation unlike DE exposed animals. In a related series of studies, the vasoconstrictive effects of the volatile organic components of DE were determined ex vivo using mouse mesenteric arteries and veins. DE at concentrations enhanced vasoconstriction and the vasoconstrictive effects of ET-1 were significantly potentiated by DE in both tissue types. Furthermore, mesenteric vessels exposed to a combined acetaldehyde, formaldehyde, acroleine, hexadecane and octadecane saline solution at concentrations found in DE-exposed saline also had enhanced ET-1 vasoconstriction. However, the individual compounds did not replicate these effects. These data suggest that exposure to the complex components of DE may induce venous congestion in susceptible subjects through enhanced vasoconstrictive due to vascular oxidative stress.

Increased Bleeding Tendency in Patients with Hematological Malignancies

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Patients with hematological malignancies (AML, ALL, multiple myeloma, B and T cells lymphoma) may often develop thrombocytopenia attributed to chemotherapy, to cancer, and to infection; they also have an increased risk of bleeding. The degree of bleeding severity includes mostly moderate bleeding sites and a low platelet count; in particular, in older patients the pathogenesis of this phenomenon is complex and it is related to platelet production, to the increased platelet destruction (i.e. hypersplenism), to abnormalities in platelet function, to circulating inhibitors or anticoagulants, and it may finally cause disseminated intravascular coagulation (DIC); hyperfibrinolysis and proteolysis were frequently associated. Clinically significant bleeding occurs in approximately 8%–12% of patients and are often associated with cytogenetic abnormalities involving chromosomes 7q (7q22, 7q32–34), 5q (5q11.1q34-21), 9p and 13q, trisomy 8. Translocation tAML/ETO from t(8;21), PML/RARA fusion transcript arising from the t(15;17), and CBFB/MYH11 from inversion 16, t(11;19)q22-23, t(11;19)(q23.p13). Cytogenetic data suggest that trisomy 8 has a “rare event” because the mutation requires early identification to permit a useful tool for predicting which patients are at risk of bleeding or relapse. In all these cases, these markers have a great prognostic significance. Patients with visible multiple petechiae, or ecchymosis, or multiple teleangiectasia of the skin, recurrent bleeding, epistaxis, hematuria, or gastrointestinal hemorrhage have been studied with particular attention (platelet count, PT, FTT, fibrinogen, FDP, D-Dimer, TAT, AT-III, TM, antiphospholipid activity). The management of bleeding disorders is based on two different options; recently two recombinant growth factors from the von Willebrand factor (vWF) family have been added to the therapeutic armamentarium of patients with a low platelet count below about 5; otherwise a platelet transfusion may be needed when platelet counts lower below 20,000/mmc.
blood TF is a contributing factor to venous thrombosis following TKA. Methods: Fifteen male subjects who had unilateral TKA gave written, informed consent. Venous blood samples were obtained before surgery, 5 min following tourniquet release and daily until hospital discharge at day 4–7. All patients received prophylactic dalteparin and none developed VTE. Platelets and mononuclear cells were isolated from EDTA anticoagulated blood by differential centrifugation. A modified thrombin time (PTT) assay was used to quantify TF procoagulant activity. Intron variant was employed as the PT calibration standard. The encrypted TF procoagulant activity was fully expressed by pretreatment with 20 μM laminin in the presence of 12.5 μM CaCl2 and 5 nM activated factor VII. Results: Blood mononuclear TF activity increased following TKA, peaked at 3 days and returned to baseline at 4 days after surgery. Platelet TF activity did not change significantly. The peak rise in TF activity preceded the median time to diagnosis of venous thrombosis following TKA (7 days) observed in previously reported studies. Conclusion: Increased mononuclear cell-derived TF activity is a delayed response to TKA which is predominantly independent of acute tissue injury. The close temporal relationship of the rise in TF to the occurrence of VTE strongly suggests that blood-borne TF contributes to VTE following TKA.

CONCLUSIONS

Changes in Activated Factor XII Type A from Admission to Day 4 Predict Recurrent Tn Positive Cardiac Events Following Admission for Myocardial Infarction

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Background: Recent research has demonstrated that in-vivo Xla exists in several different forms, and that activated Factor XII type A (XIIaA) concentrations are strongly predictive of mortality. The study’s aim was to assess the ability of changes in concentration of XIIaA from admission to day 4 in predicting outcome in patients admitted with MI. Methods: Quantiles were established based on the change in XIIaA between admission and day 4. New Tn positive events and all cause mortality were correlated to the change in XIIaA in blood samples taken at admission and 4 days post admission and compared at 30 days, 6, 12, and 24 months follow-up in 319 patients admitted with MI. Analyses of XIIaA were performed on citrated blood samples collected according to the Leiden fibrinolysis study. Changes in XIIaA between admission and day 4 post-MI were associated with risk for changes in XIIaA at admission and 4 days post-MI in predicting outcome in patients admitted with MI.

Changes in XIIaA between admission and day 4 post-MI were associated with risk for changes in XIIaA at admission and 4 days post-MI in predicting outcome in patients admitted with MI.

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Serum Adiponectin Levels Associate with Tissue-type Plasminogen Activator Activity in Patients with Advanced Carotid Atherosclerosis

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INTRODUCTION Adiponectin is an adipocyte-derived bioactive substance with multiple functions including antiatherogenic properties. Recently, hypoadiponectinemia has been associated with platelet activation in carotid atherosclerosis. OBJECTIVE The objective of this study was to examine the relationship of serum adiponectin levels with advanced carotid atherosclerosis and detailed clinical data including variables of fibrinolytic activity. METHODS The Helsinki Carotid Endarterectomy Study included 92 consecutive patients with severe operable symptomatic (stroke or TIA) or asymptomatic carotid stenosis (=70%, NASCET). Blood samples were collected before surgery and processed immediately for the determination of coagulation and fibrinolysis-associated variables. Tissue-type plasminogen activator (tPA) antigen, -activity and plasminogen activator inhibitor-1 (PAI-1) antigen and activity were measured from plasma samples collected according to the Leiden fibrinolysis study. Serum adiponectin levels were measured using a commercial enzyme-linked immunosorbent assay. RESULTS Univariate correlations confirmed the previous positive correlations of adiponectin with female gender, HDL, age and negative correlations with BMI, triglycerides, PAI-1 antigen and activity. In addition adiponectin levels showed consistent association with IPA antigen (r = 0.38) and even more strongly with IPA activity (r = 0.550) (backward regression model). Patients with asymptomatic and symptomatic carotid stenosis did not show significant differences in adiponectin levels although patients with no previous history of cerebrovascular symptoms tended to have higher adiponectin levels (p = 0.10).

CONCLUSIONS Adiponectin does not seem to be a risk factor for unstable carotid atherosclerosis but it showed strong association with IPA activity, the surrogate marker of fibrinolytic activity, in patients with advanced carotid atherosclerosis.

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DG-041, a Novel Antiplatelet Agent, Is a Potent and Selective Antagonist of the EP3 Receptor for Prostaglandin E2

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Background: Genetic studies in Iceland find that DNA variants in the EP3 receptor for prostaglandin E2 are associated with increased risk for peripheral arterial disease (PAD), myocardial infarction and stroke. The EP3 receptor is expressed on platelets, but not in the vessel wall suggesting that aberrant signaling through the platelet PGH2/PGE2 pathway may play a role in arteriolar thrombotic disease. PG22 signaling through EP3 platelets amplifies platelet responses to platelet co-aggregants but has no effect in the absence of a co-aggregant. METHODS: DG-041 is a novel inhibitor of cyclooxygenase-1 (COX1) and COX2, and a potent, selective EP3 receptor antagonist. RESULTS: DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist. DG-041 inhibited thromboxane A2, PGE2 and PGF2α production in vitro with IC50 values of 10 nM, 1 μM and 5 μM, respectively. DG-041 is a potent and selective EP3 receptor antagonist.
and other endogenous ligands) may increase thrombus formation and exacerbate the development of atherosclerosis. We confirmed that TL4 agonists activate platelets and increase ROS formation. We also found that LPS, and the TL2R agonist bacterial lipoprotein, induced platelet P-selectin expression, and that ROS production was partially blocked by pretreatment with antioxidant Trolox (water-soluble vitamin E analog). LPS also potentiated platelet aggregation and maximized the oxidative stress. Thus, the failure to test the postulate that TL4- and TL2R agonists may have induced expression of ascorbyl-2-succinic-cyclooxygenase-2 (COX-2). We detected COX-2 in agonist-treated platelets using real-time RT-PCR, confocal microscopy, FACS, and Western blot. Trolox treatment significantly down-regulated COX-2 protein expression in LPS-activated platelets. TXA2 synthesis in LPS-treated cells was less sensitive to aspirin, but sensitive to COX-2 inhibitor NS398. These observations suggest that the induction of COX-2 in platelets by the agonists of TL4 and TL2R mediate aspirin-insensitive production of TXA2, and so upregulation of COX-2 in platelets may be one mechanism leading to aspirin resistance. Application of antioxidants together with COX-2 inhibitors may be useful in the treatment of acquired aspirin resistance.

**The Role of Akt in Glycoprotein Ib-IX–Mediated Platelet Activation**

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Under high shear rate flow conditions, initial platelet adhesion at the site of vascular injury is amplified by the interaction between von Willebrand factor (vWF) and its receptor, the glycoprotein Ib-IX (GPIb-IX) complex. This interaction also initiates a signaling cascade, leading to activation of the platelet integrin, β3. The signaling mechanism of GPIb-IX is not fully understood. The cytoplasmic domain of GPIb-IX has been suggested to interact with signaling molecules and to promote phosphorylation of 3-kinase (PI3-kinase), which has been shown to be involved in the GPIb-IX signaling. An important signaling molecule that is activated by PI3-kinase is Akt. Thus, to understand the downstream signaling pathway of GPIb-IX signaling, we investigated the role of protein kinase, Akt, in vWF-induced, GPIb-IX-mediated platelet activation. We showed that GPIb-IX-vWF interaction-dependent platelet aggregation induced by botrocetin or bortezomib was impaired in Akt-deficient mouse platelets or human platelets treated with an Akt inhibitor, SH-6. In contrast, botrocetin-induced vWF binding to platelets or platelet agglutination was not significantly affected. Similarly, GPIb-IX and integrin-dependent platelet spreading on vWF was unaffected in Akt-deficient or Akt inhibitor-treated platelets.

Under high shear rate flow conditions induced by the cone-plate rheometer, GPIb-IX- and integrin-dependent stable platelet adhesion on immobilized vWF was attenuated in Akt-deficient or SH-6 treated platelets. Thus, Akt is important in GPIb-IX and integrin-dependent platelet adhesion, spreading and aggregation. The role of Akt-1 is independent of integrin outside-in signaling, because Akt inhibitor-treated or Akt-1 null platelets are not different from control platelets in spreading on immobilized fibronectin, which requires integrin outside-in signaling but not integrin activation. Thus, Akt-1 plays an important role in GPIb-IX-mediated platelet activation signaling, independent of integrin outside-in signaling. Our results support the hypothesis that GPIb-IX signaling is necessary for optimal platelet aggregation and vWF-induced the P3K-dependent Akt phosphorylation. Taken together, our data indicate an important role for Akt in GPIb-IX-mediated platelet activation signaling.
Nitric Oxide Release from Pulmonary and Coronary Vasculature with the Use of Intravenous Iloprost and Nitroglycerin in Diabetic Patients Undergoing Valvular Heart Surgery

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Introduction: The aim of this study is to compare the effects of intravenous iloprost and nitroglycerin in patients with type II diabetes mellitus undergoing valvular heart surgery. Methods: Twenty five patients undergoing valvular replacement with pulmonary hyperten-
sion > 25 mmHg were randomized to be given iloprost or nitroglycerin via a central pulmonary catheter, and the levels of nitrite/nitrate were evaluated before incision (T1) and 20 minutes after incision in a coronary bypass. Aortic, coronary, and pulmonary mixed venous blood samples were taken at the T1 and T4 time periods and the release of nitric oxide from the coronary vasculature was determined by the difference between the aortic and coronary sinus concentrations of nitrite and nitrate. Results: Before application of aortic cross clamp, at T1, the levels of nitrite/nitrate from the coronary vasculature were similar in both iloprost (group 1) and nitroglycerin (group 2) groups (18.43 ±M/mL versus 13.8 ±M/mL, median, p < 0.05). However, after the removal of the cross-clamp, a significant increase in nitric oxide is observed in the group 2 at T4 comparing to group 1 (26.1 ±M/mL versus 34.2 ±M/mL, p < 0.05). Conclusion: This study has shown that in patients with type II diabetes mellitus undergoing valvular heart surgery the iloprost group did not show an increase in the release of nitric oxide from the coronary vascular bed after aortic cross clamp and during reperfusion period. This finding suggests that iloprost and nitroglycerin act via different mechanisms on vascular smooth muscle cells during their use for pulmonary hypertension.

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Reversal of Hypercholesterolemia Promotes Progression of Atherosclerosis and Protects Against Arterial Thrombosis in Mice

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Introduction: Hypercholesterolemia promotes atherosclerosis and increases thrombotic risk, but little is known about the effect of cholesterol lowering on susceptibility to experimental thrombosis. Hypothesis: We tested the hypothesis that reversal of hypercholesterolemia induces regression of atherosclerosis and restores normal susceptibility to carotid artery thrombosis in Reverse mice. Methods and Results: Male Reverse (LdrI Apob<sup>-/-</sup> Mtpp1<sup>-/-</sup> Mx1-Cre<sup>-/-</sup>) were fed a high fat diet and studied at 5 or 6 months of age (total cholesterol (TC) 784 ±66 and 726±65 mg/dL, respectively). To prevent hypercholesterolemia, Reverse mice were injected with polynicosinic-polycytidylic acid (pI-pC) at one month of age to induce Mx1-Cre and switch off hepatic Mtp gene expression, fed a control diet, and studied at 5 or 9 months of age (TC 63±10 and 70.7±6 mg/dL, respectively). To reverse hypercholesterolemia, Reverse mice were fed a high fat diet until 5 months of age, then injected with pI-pC and switched to a control diet, and studied at 9 months of age (TC 75±12 mg/dL). Atherosclerotic lesion area was measured in aortic sinus cross-sections and in face preparations of the thoracic aorta. At both sites, lesion area was greater in the hypercholesterolemia group compared with the normocholesterolemic group at P < 0.05 and greater at 9 months compared with 5 months (P < 0.05). In the reversed group, aortic lesion size at 9 months was similar to the hypercholesterolemia group at 5 months, but was lower than the hypercholesterol-
olemia group at 9 months (P < 0.05), indicating that atherosclerosis did not progress between 5 and 9 months. Susceptibility to carotid artery thrombosis was measured using a photochemical injury model. Thrombotic occlusion occurred more rapidly in the hypercholes-
terolemia group than in the normocholesterolemic group at both 5 months (17±8 vs. 46.5±12 minutes; P < 0.05) and 9 months (8.1± vs. 4.5±9 minutes; P < 0.05). Accelerated thrombosis was not observed in normocholesterolemic hypercholesterolemia (31±12 minutes; P < 0.05 vs. hypercholesterolemia group). Conclusions: Reversal of hypercholesterolemia normalizes thrombotic susceptibility in atherosclerotic mice.

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Induction of Thrombotic Plaque Erosion in Balloon-injured Arteries of Atherosclerotic RABBTS

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Objective: Thrombus erosion triggers thrombosis, serving as a vital pathologic basis of coronary thrombotic events. This research sought to establish a model of thrombotic plaque erosion in atherosclerotic rabbit aortas by stauorospore-triggered endothelial apoptosis. Methods: Atherosclerotic plaques were established in 33 New Zealand rabbits by post-balloon-injury high-cholesterol feeding for 3 months. The animals were randomized into two groups and their plaque-rich abdominal aortas were induced with staurospore (group A, n=24) or saline (group B, n=12). Group A was randomized into group A1 (n=12) and group A2 (n=12); they were triggered with 5×10<sup>-10</sup> or 1×10<sup>-9</sup> mol/L stauorospore, respectively. Three days after induction, thrombotic score was performed according to angiography (angiographic obvious thrombosis was defined as score 3–4). Serial sections of the induced segments were conducted to count incidence of histological thrombosis. Results: Angiographic thrombosis rates relative to group B, the incidence of obvious thrombosis was higher in groups A (P<0.05), in which it was higher for group A1 (58.3%, P<0.01) but not for A2 (16.7%, P<0.05). Serial sections of the processed aortas revealed that there were more histological thrombosis in group A and A1, than in group B (P<0.01, respectively). Apoptosis scores according to TUNEL staining were higher in groups A and A1 than in group B (P<0.01, respectively). Pathological analysis showed that, non-ruptured and smooth-muscle-cell-rich fibrous caps at plaque/thrombi interfaces were observed in serial sections stained with HE. Oil Red O and α-actin, and endothelium integrity according to CD31 staining were lower in groups A and A1 than in group B (P<0.01, respectively). Apoptosis scores according to TUNEL staining were higher in groups A and A1 than in group B (P<0.01, respectively). Logistic analysis demonstrated that endothelial apoptosis was an independent risk factor of histological thrombosis and angiographic obvious thrombosis (OR=15.22, P<0.01; OR=45.74, P<0.01). Conclusion: A thrombotic plaque erosion model can be successfully established by perfusing staurospore into atherosclerotic abdominal aortas; and 5×10<sup>-10</sup>mol/L stauorospore appears to be more suitable with a higher angiographic obvious thrombosis. In addition, endothelial apoptosis may play a critical role in thrombotic plaque erosion.

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Bilirubin Oxidation Products Elicit Significantly Elevated ThromboXase Production in Rat Brain

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Bilirubin Oxidation Products (BxOEs) have been found in the CSF of patients with cerebral vasospasm after SAH, as well as in hemorhatic and perihemorrhatic brain of animal models of ICH. We have found that BxOEs are vasoactive, both in vitro and in vivo, and so wanted to investigate possible effects of Boxes on the inflammatory responses of the brain itself. Contralateral animals were anesthesiazed and perfused with 0.9% saline. The brains were removed and flash-frozen. L autologous blood was infused into the right cerebral cortex. The animal was allowed to recover for 24 hours, then the brain was harvested as for the control animal. Boxes: a cranial window was opened over the right cerebral hemisphere and 25µL of 23µM BxOEs was applied directly to the surface of the brain. The animal was allowed to recover for 24 hours, then the brain was harvested as for the control animal. Contralateral cortex (from ICH and Boxes brains) was homogenized and assayed for Thromboxane B<sub>2</sub> using small-molecule ALKS inhibitor in a vascular fibrosis model and suggest the potential therapeutic application of these inhibitors in vascular fibrosis.
an EIA kit (Cayman Chemicals). Total protein was assayed using the BCA method (Pierce). The results indicate that B0X14 induces TX production in the brain, in the absence of thrombin and other blood components normally present in hemorrhagic stroke. This suggests a role for B0X14 in other complications (than vasospasm) following hemorrhagic stroke such as edema, inflammation and immune responses. It may also hint at a putative role for therapeutic use of inhibitors of TX production, such as COX-2 inhibitors.

5-Amino-4-imidazole Carboxamide Riboside Inhibits Tissue Factor Induction in Endothelial Cells and Monocytes

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AMP-activated protein kinase (AMPK) is tightly regulated by the ratio of intracellular AMP/ATP and plays a central role in regulation of energy homeostasis under metabolic stress. Recent studies link AMPK to an anti-inflammatory effect such as inhibition of cytokine production and adhesion molecule expression. In this study, we used 5-amino-4-imidazole carboxamide riboside (AICAR), one of AMPK activators to investigate whether AMPK plays a role in anti-thrombotic effect. Tissue factor (TF), a critical initiator of physiologic and pathologic coagulation, plays an important role in initiation and propagating thrombus formation in heart attack and stroke. In an in vitro assay, the clotting activity of TF in human umbilical vein endothelial cells (HUVEC) and peripheral blood mononuclear cells were induced by physiologic agonist LPS, TNF-a and IL-1b. Pretreatment of cells with AICAR inhibited clotting activity by ~90%. Inhibition of TF clotting activity by AICAR appears dose and time-dependent. Suppression of TF clotting activity correlated a decrease of TF expression at protein and mRNA levels. ZM 241385, a specific adenosine A2a receptor antagonist, did not block the effect of AICAR on TF suppression, indicating inhibition of TF by AICAR was not due to adenosine production and A2a occupancy. We are using AMPK knockout or knockdown cells in our study. The consequent results will provide information on the extent of AMPK dependency in AMPK-mediated TF inhibition.

Nocturnal Oxygen Desaturation and the Prevalence of Metabolic Syndrome

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Objective: Many studies in the past have identified an association between obstructive sleep apnea (OSA) and the elements of MS. Recently some studies have found a direct association between the severity of OSA and the prevalence of MS in a community subpopulation diagnosed with obstructive sleep apnea. Methods: A cross sectional retrospective analysis of the data of 419 patients was performed at a community sleep disorders diagnostic center. MS was considered to be present in patients if three out of the following criteria were met: hypertension, diabetes, hyperlipidemia and a body mass index of greater than 30. Nocturnal oxygen desaturation was considered to be significant if the oxygen concentration during the sleep study desaturated below 90%. Results: A total of 22 patients were identified with MS. The NCSS and PASS statistical software was used for the analysis. A correlation value of 0.93 and a p value of 0.0009 was found. Although the correlation value was not significant, the study found that in this subpopulation with OSA, in general, a higher percentage of total sleep time spent below 90% was accordingly associated with a higher number of cases with MS (graph 1). Conclusions: The study found a dose dependent association between the severity of nocturnal oxygen desaturation and the prevalence of MS. Whether or not the reversal of nocturnal oxygen desaturation through CPAP treatment corrects the syndrome needs to be tested in a large multi center prospective study.

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Hyperglycemia-Induced Cell Growth and Gene Expression via Serum Response Element Through Pkcγ, Rhoa, and Rho-Kinase in Vascular Smooth Muscle Cells

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The impressive correlation between cardiovascular disease and alterations in glucose metabolism has raised likelihood that atherosclerosis, heart failure and type 2 diabetes may share common antecedents. Postprandial hyperglycemia has been shown the important role for onset and development of heart failure and cerebral infarction by several large-scale clinical trials. Recently, chronic hyperglycemia has been reported to enhance the vasocostructor response by Rho-kinase and PKC. Furthermore, oral PKCγ selective inhibitor has been reported to show the effective therapy for the cardiovascular complications of diabetes. We have reported Phenylephrine showed enhancement of vasocostructor response in a spontaneous diabetes mellitus model, OLETF (Otsuka-Lon-Evame-Takushima fatty ) rat. However, the mechanism of hyperglycemia on these reactions, especially the influence to signal transduction pathway by hyperglycemia has not been well understood. Therefore, we examined the effect of hyperglycemia on cell growth and gene expression in rat aorta smooth-muscle cells (RASMcs). Hyperglycemia accelerated the growth of RASMcs with concentration dependent manner. Furthermore, c-fos gene expression was also increased by hyperglycemia. Phenyl- ephrine activated c-fos gene expression. Hyperglycemia augmented Phenylephrine-induced c-fos gene expression synergistically with dose dependent manner. The deletion analysis revealed c-fos serum response element (SRE) accounts for c-fos gene expression. PKCγ, RhoA, and Rho-kinase were involved in this signal transduction pathway. Furthermore, PKCγ-activated c-fos SRE expression was inhibited by RhoA and Rho-kinase. These results indicate RhoA and Rho-K is downstream molecules of protein kinase C (PKC γ). HMG-CoA reductase inhibitor, Fluvastatin inhibited hyperglycemia- augmented these reactions by inhibi- tion of RhoA. Furthermore, calclytic domain mutant of PKCγ also inhibited these reactions. Hyperglycemia itself increased the cell growth and gene expression. Furthermore, it also modifies and augments the cell growth and gene expression by α1-AR-mediated stimulation. Statin and PKCγ inhibitor might be effective for hyperglycemia-induced cardiovascular dysfunction.

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Red Blood Cell Fatty Acid Composition and the Metabolic Syndrome: NHLBI GOLDN Study

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Different fatty acids may vary in their effect on the metabolic syndrome (MeS). We tested whether fatty acids measured in red blood cells (RBC) are associated with the MeS or its components. Men (n = 466, 49 ± 16 y) and women (n = 535, 49 ± 16 y) from 187 families from Utah and Minnesota were studied as part of the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. Fatty acids in RBC were measured with gas chromatography while data on confounders were obtained from interviewer-administered questionnaires. The prevalence of the MeS as defined by the updated Adult Treatment Panel III criteria was 37.0% in Utah and 39.8% in Minnesota (P < 0.05). In a multivariate model that included four fatty acid classes, age, sex, physical activity, alcohol intake, smoking and pedigree (modeled as a random effect), polyunsaturated and saturated fatty acids were significantly associated with the prevalence of the MeS (Table 1). We observed significant (P < 0.05) correlations between fatty acid classes and components of the MeS (data not shown). Saturated fat (r = 0.10), monounsaturated fat (r = 0.12) and polyunsaturated fat (r = -0.11) were significantly correlated (P < 0.05) with fasting insulin. In conclusion, polyunsaturated fatty acids were inversely associated with the MeS while saturated fatty acids were positively associated with the MeS probably through their effect on lipids, insulin and systolic blood pressure. These data suggest that RBC fatty acid profiles could be a good marker for the metabolic syndrome.

Table 1. Odds Ratios (95% CI) for Fatty Acid Classes and the Metabolic Syndrome

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>1.00</td>
<td>0.69 (0.46–1.06)</td>
<td>0.63 (0.40–0.99)</td>
<td>0.40 (0.24–0.65)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>1.00</td>
<td>1.25 (0.79–1.99)</td>
<td>1.44 (0.92–2.27)</td>
<td>1.62 (1.02–2.62)</td>
<td>0.04</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>1.00</td>
<td>0.78 (0.51–1.20)</td>
<td>0.72 (0.46–1.13)</td>
<td>0.81 (0.50–1.32)</td>
<td>0.39</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>1.00</td>
<td>1.25 (0.83–1.90)</td>
<td>1.26 (0.82–1.94)</td>
<td>0.96 (0.59–1.56)</td>
<td>0.89</td>
</tr>
</tbody>
</table>
comparison between adiponectin and leptin in relation to metabolic syndrome in japanese women

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background: Two representative adipokines, adiponectin and leptin, have been suggested to play important roles in the pathogenesis of metabolic syndrome (mets). however, little information exists on their respective risk in relation to mets components in women.

objective: To investigate the respective associations of adiponectin and leptin with each mets component and its clustering.

Methods: we studied 769 middle-aged japanese women without a history of cancer or cardiovascular disease. Since it was less likely that changes in these adipokines cause an increased incidence, we focused on the association of adiponectin or leptin with 4 mets components other than central obesity. thus, we computed odds ratios (ORs) in the presence of the each mets component or its clustering (≥2 components) according to a 1-SD decrease in log-adiponectin and a 1-SD increase in log-leptin with multivariate logistic regression analyses, in which both adipokines were simultaneously entered as explanatory variables.

results: Interestingly, adiponectin had a closer association than leptin with low high-density lipoprotein cholesterol (HDL-C) and hyperglycemia (Table). in contrast, the association with elevated blood pressure was significant only for leptin. hypertriglycerideemia was significantly associated with both adipokines. as a result, both adipokines were independently and significantly associated with the clustering of mets components.

conclusion: Although both adiponectin and leptin were independently associated with mets, their associations with each mets component were distinctive. Specifically, decreased adiponectin seemed to play a greater role in the development of HDL-C and the elevation of glucose. so did increased leptin in the elevation of blood pressure. these data may have important implications both for inferring the etiological role of these adipokines in causing mets and for introducing them as targets for mets treatment.

Table: Comparison of adiponectin and leptin in relation to mets

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Adiponectin</th>
<th>Leptin</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low HDL-C</td>
<td>1.84 (1.40–2.42)</td>
<td>&lt;0.001</td>
<td>1.28 (0.94–1.71)</td>
<td>0.12</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>1.41 (1.11–1.79)</td>
<td>0.005</td>
<td>1.89 (1.43–2.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>1.31 (1.00–1.71)</td>
<td>0.051</td>
<td>1.02 (0.76–1.37)</td>
<td>0.91</td>
</tr>
<tr>
<td>Elevated blood pressure</td>
<td>1.08 (0.91–1.28)</td>
<td>0.41</td>
<td>1.41 (1.17–1.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>1.52 (1.20–1.93)</td>
<td>0.001</td>
<td>1.62 (1.27–2.19)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*ORs (95% CI) by a 1-SD decrease and increase of log adiponectin and log leptin, respectively, adjusted for age and smoking.

Thiazolidinediones attenuate the angiotensin Il-mediated enhanced vascular responses in high fat diet–fed rats by altering the characteristics of L-type calcium channels

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Background/rationale: insulin resistance has emerged as a mechanism leading to diabetes mellitus and hypertension. however, insulin sensitizers such as pioglitazone and rosiglitazone, [peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists] are reported to reduce blood pressure (BP) in hypertensive models by altering L-type calcium channel functions.

Objective/hypothesis: To establish the cause and effect relationship of insulin resistance and vasculopathy and find the role of L-type calcium channels. Methods: Ang II-induced contractions were studied isometrically in thoracic aortic rings isolated from control and high fat diet (HFD) fed rats. to evaluate the involvement of L-type calcium channels in Ang II-mediated contraction, cumulative concentration response curves (OCR) to Ang II was constructed in the presence of various concentrations (0.01 nM-1 μM) of nifedipine (dihydropyridine sensitive L-type calcium channel blocker) and the IC50 was estimated.

results: the rats fed with HFD for four weeks exhibited the conglomeration of characteristic features of insulin resistance syndrome, such as obesity, hyperinsulinemia, mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia, glucose intolerance and hypertension. maximal contractile response (Emax) to Ang II was increased in HFD fed rats as compared to control rats. in addition, Bmax values and affinity of Ang II receptors and L-type calcium channels are increased, respectively. nifedipine dose-dependently blocked the Ang II-induced contractions in a noncompetitive manner and its IC50 was significantly lower in aortic rings from HFD fed rats (8.87 ± 0.17) compared (P<0.05) to control (9.78 ± 0.18) rats. Rosiglitazone and pioglitazone treatment for 14 days restored IC50 values (P<0.05) comparable to that of control (8.88 ± 0.26 & 8.62 ± 0.34, respectively). conclusions: Thiazolidinediones attenuate the development of hypertension and improves the vascular dysfunction induced by Ang II by altering the L-type calcium channel functions in HFD fed rats.

Repetitive Glucose Fluctuations Accelerate Macrophage Adhesion to Endothelial Cells and Formation of Arteriosclerotic Lesions in Apolipoprotein E-Deficient Mice

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background: Postprandial hyperglycemia is regarded as an independent risk factor for the progression of atherosclerosis. Recently, we demonstrated that repetitive postprandial hyperglycemia per se induced monocyte adhesion to endothelial cells by using a new in vitro method.
Carboxyl Ester Lipase Deficiency Exacerbates Dietary Lipid Absorption Abnormalities and Resistance to Diet-Induced Obesity in Pancreatic Triglyceride Lipase Knockout Mice

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Pancreatic triglyceride lipase (PTL) is generally accepted as the principal enzyme involved in the hydrolysis of dietary fat, thereby mediating its absorption. However, trisglyceric (TAG) absorption was minimally altered in PTL/-/- mice. This study tested the hypothesis that the compensatory enzyme in TAG hydrolysis and absorption in PTL/-/- mice is carboxyl ester lipase (CEL). PTL/CEL/-/- double knockout mice were generated via crossbreeding. The ability of TAG absorption was assessed. Net TAG absorption was reduced from 91.5±0.7% in wild type mice to 80.1±3.7% in PTL/-/- mice (p<0.05). Strikingly, this was reduced to 61.1±3.8% in PTL/CEL/-/- mice (p<0.01). Defective free cholesterol absorption reported previously in PTL/-/- mice was confirmed in this study. Cholesterol absorption in PTL/-/- mice was 41% less than wild type (p<0.05), but this difference was not observed in PTL/CEL/-/- mice (p<0.01). Additionally, absorption of retinyl palmitate from the intestinal tract to the plasma was reduced by 45% and 60% in PTL/-/- and PTL/CEL/-/- mice (p<0.05) respectively. On a high fat diet, food intake was not different between mice with the various genotypes. After 15 weeks of feeding the high fat diet, body weight of CEL/-/- mice increased by 10% compared to wild type, but PTL/-/- and PTL/CEL/-/- mice gained an average of 6.2 g and 8.6 g less respectively (p<0.01). Body composition analysis showed that both PTL and PTL/CEL mice consuming the high fat diet carry less fat and more lean mass than wild type mice. In summary, we show that PTL and CEL together are responsible for a major portion of dietary fat and fat-soluble vitamin absorption. Our results support free cholesterol absorption requiring efficient TAG hydrolysis in the proximal gut. Although the residual 50–60% of dietary TAG absorption in PTL/CEL/-/- mice suggests an additional TAG lipase exists in the gut, deficiency of both PTL and CEL confers protection against diet-induced obesity. Thus specific inhibition of PTL or CEL may be a therapeutic approach to diet-induced obesity without fat-soluble vitamin deficiency or steatohepatitis associated with total inhibition of lipolytic enzymes in the intestinal tract.

Telmisartan Improves Metabolic Syndrome in Addition to Its Vascular Protection in High-fat Diet–Fed Insulin-Resistant Rats

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Background/rationale:– Increased vascular contraction by angiotensin II and decreased relaxation by impaired endothelial function causes the development of vascular dysfunction in insulin resistance (IR). However, peroxisome proliferator activated receptor γ (PPAR γ) activating agents have been documented for vascular protection by unclear mechanisms. Hence, the inhibition of RAS and activation of PPAR γ simultaneously could have the beneficial effects in IR.

Methods:–Angiotensin II (Ang II) and acetylcholine (ACH) mediated vascular responses were studied simultaneously in thoracic aortic rings isolated from normal and high fat diet (HFD) fed rats. Other biochemical parameters were analyzed by commercially available spectrophotometric kits. Receptor radioligand binding studies for Ang II receptors were carried by [3H]-Ang II. Telmisartan [AT1 blocker & partial PPAR γ agonist], pioglitazone and losartan were administered orally for 14 days prior to all the experiments.

Results:– HFD-fed rats exhibited characteristic features of metabolic syndrome (MetS) viz., obesity, hyperinsulinemia, mild hyperglycemia, hypertyroidism, hypercholesterolemia, glucose intolerance and hypertension.

Vascular dysfunction was evident from the increased maximal contractile response to Ang II and decreased vascular relaxation to ACh in thoracic aortic rings. Angiotensin receptor blocker (ARB), losartan improved the vascular dysfunction without altering metabolic parameters.

Pioglitazone improved the metabolic parameters with a marginal improvement of vascular parameters. However, the telmisartan produced similar kind of effects of combination of pioglitazone and losartan including down regulated AT, receptors with an addition of weight reduction potential.

Conclusion:– Telmisartan improves abnormal metabolic and vascular profile in addition of weight reduction potential in MetS. Due to coexistence of metabolic syndrome and CVDs, the availability of the multifunctional dual pharmacophores like telmisartan can treat more than just blood pressure or metabolic disturbances which could be of considerable clinical value.

12/15-Lipoxygenase Activity Reduces ABCA1-Mediated Cholesterol Efflux Through Increased ABCG1 Phosphorylation in Macrophages

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A key step in early atherogenesis is the formation of lipid-laden foam cells. Oxidation of low-density lipoprotein (LDL) is a critical step in foam cell formation. There are multiple mechanisms for in vivo LDL oxidation, and the enzyme 12/15-lipoxygenase (12/15LO) contributes to this oxidation. 12/15LO incorporates molecular oxygen in a stereospecific manner to yield 12- and 15(S)-hydroxyeicosatetraenoic acids (12SHETE/15SHETE). Disruption of the 12/15LO gene protects mice from atherosclerotic lesion formation, while transgenic mice that overexpress 12/15LO are more prone to lesion development. We recently found that J774 macrophages that stably overexpress leukocyte 12/15LO (Plox-86) while transgenic mice that overexpress 12/15LO are more prone to lesion development. We recently found that J774 macrophages that stably overexpress leukocyte 12/15LO (Plox-86) have a 35% reduction in cholesterol efflux to HDL compared to mock-transfected cells (Mock 4).

Treatment of control J774 cells or Mock 4 with 12/15LO product 12SHETE (500mM, 24 hours) also reduced efflux to HDL (15% reduction in J774 and 12SHETE and 34% reduction in Mock 4 + 12SHETE). Concurrently with the reduction in efflux, we saw a 2-fold reduction in protein expression of the ABC transporter ABCA1 followed by a 2-fold reduction in expression of macrophages with 12SHETE or in Plox-86 12/15LO-expressing macrophages, indicating that the reduction in...
cholesterol efflux is due to reduced ABCG1 expression. However, we found no changes in ABCG1 mRNA expression in these macrophages by 12/15LO products, suggesting that the decrease in ABCG1 protein observed by 12/15LO is not caused by transcriptional changes in ABCG1 mRNA. Thus, we hypothesized that ABCG1 is regulated post-translationally by 12/15LO products. We examined ABCG1 phosphorylation by immunoprecipitation (IP). IP studies revealed increased tyrosine and threonine phosphorylation of ABCG1 following treatment with 12(S)HETE (500nM, 24 hours) or in Plox-86 12/15LO-expressing cells. This protein phosphorylation may lead to degradation of ABCG1, thereby increasing macrophage foam cell formation via reducing cholesterol efflux from the macrophage. Thus, these data provide evidence for a novel role of 12/15LO in promoting foam cell formation. Understanding the role of ABCG1 in macrophages will aid in developing beneficial therapies that target genes involved in cholesterol metabolism.

P278 The Role of ES-4 as a Neutral Cholesterol Ester Hydrolase in Hepatic Cells
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The ability of cells to control cholesterol levels is a balance between the de novo synthesis and uptake of cholesterol versus secretion and efflux of cholesterol. Excess cholesterol stored as can be toxic and is stored as cholesterol esters (CE), CE can supply free cholesterol (FC) to be utilized for synthesis of steroids, bile acids or for efflux from the cell, through the action of non-lysosomal CE hydrolases. These hydrolyase(s) have been given the general name neutral cholesterol ester hydrolase (NCEH). Our data indicate that the protein esterase 4 (ES-4) is a strong candidate as a rat hepatic NCEH. We have determined in rat hepatocyte effects of the modulating ES-4 levels using siRNA and overexpression, on total cholesterol, CE, and FC levels and FC efflux. Rat hepatoma McA cells overexpressing ES-4 showed decreased CE levels compared to control cells indicating that ES-4 can hydrolyze CE in intact cells. Also in cells where ES-4 levels were suppressed by 75% by siRNA, there was increased (by 50%) CE levels. To determine whether the change in CE was from ES-4 mediated hydrolysis, we used radiolabeled H2-CE in McA cells with varying ES-4 levels. The results showed that in ES-4-expressing McA cells, CE hydrolysis was increased by 40% and these results strongly indicate that ES-4 is a NCE in hepatic cells. Currently we are evaluating the role of ES-4 in cholesterol metabolism using primary rat hepatocytes.

P279 Identification of a Novel Lipid Efflux Defect That Is Not Due to Mutations in the ABCA1 Gene but to Regulation of ABCA1 Protein
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Currently, HDL deficiency due to genetic causes is attributed to mutations in three genes - LCAT, Apo A1 and ABCA1. These genes, however, do not account for the majority of cases of low HDL. We have identified 42 French-Canadian subjects with severe HDL-c deficiency (< 5th percentile of the population) in whom mutations in LCAT, Apo A1 and ABCA1 have been excluded (via candidate gene, haplotyping and linkage analysis approaches). To further identify individuals in whom the low HDL phenotype is due to defective HDL synthesis, cellular lipid efflux assays were performed in skin fibroblasts from the subjects. The fibroblasts were loaded with free cholesterol and incubated with lipid-free apoA 1 (24 h for each) to stimulate cellular lipid efflux. The assay results indicated two probands in whom both cholesterol and triglyceride levels were significantly increased above normal levels with 22OH/RA stimulation, indicating that the increase in cholesterol was not due to decreased ABCA1 expression. This results strongly indicate that ES-4 is a NCEH in hepatic cells. Currently we are evaluating the role of ES-4 in cholesterol metabolism using primary rat hepatocytes.

P280 Human StarD4: Localization and Sterol Transport Characteristics of a STAR-related Lipid Transfer Protein
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StarD4 mRNA has been detected in heart, liver, lung, and kidney, and is a presumed intracellular sterol transport protein based upon its STAR domain. Objective: Characterize this novel STAR-related domain by determining its localization and its ability to transport steroids. Methods: Human StarD4 was His-tag purified and a StarD4 polyclonal antibody generated for Western analysis and immunocytochemistry. Results: Binding assays showed recombinant StarD4 was selective in its binding of cholesterol in a molar ratio of 1:1, but was unable to bind any of the other tested sterols. As we have previously observed with StarD1 and StarD5, Western analysis detected high levels of StarD4 protein in human monocytes/macrophages. Despite prior detection of high mRNA levels within liver tissue, StarD4, like StarD5, was not found in freshly isolated or cultured human hepatocytes. Immunocytochemistry localized StarD4 intracellularly to the macrophage cytosol. Cellular fractionation confirmed cytosolic localization of full length (248Da) StarD4 with a smaller (18KDa) StarD4 degradation band also detected in the mitochondrial fraction. To corroborate cholesterol binding observed with recombinant human StarD4, evidence for cholesterol esterification was also looked for following StarD4 expression in hepatocytes, i.e. cells which do not express detectable StarD4. Interestingly, StarD4 overexpression led to a ~5-fold increase in bile acid synthesis via the CYP27A1 initiated mitochondrial pathway and an increase in cholesterol ester formation. This identification was also looked for following StarD4 expression in hepatocytes, i.e. cells which do not express detectable StarD4. Interestingly, StarD4 overexpression led to a ~5-fold increase in bile acid synthesis via the CYP27A1 initiated mitochondrial pathway and an increase in cholesterol ester formation. Understanding the role of StarD4 in macrophages will aid in developing beneficial therapies that target genes involved in cholesterol metabolism.

A Novel Endoplasmic Reticulum-derived Organelle Abundantly Present in Cholesterol-Rich Human Macrophages and Expresses Elevated Acyl-CoA: Cholesterol Acyltransferase 1 Enzyme Activity
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Under hyperlipidemic condition, aortic macrophages continue to internalize modified low-density lipoprotein (LDL), and are transformed into cholesterol-rich foam cells. The modified LDL liberates free cholesterol, which is converted to enoyl acyl coenzyme A:cholesterol acyltransferase (ACAT1). These events occur during early stages of atherogenesis. Using immunoelectron microscopy, we had previously shown that, in human monocyte-derived macrophages grown under normal lipidic condition, ACAT1 is mainly located in the endoplasmic reticulum (ER); however, when these cells are overloaded with modified LDL, a significant portion of the ACAT1 signal is present in small, ER derived vesicles. To further pursue this finding, in the current work, we prepared postnuclear cell homogenates from the THP-1 macrophages, and subjected them to subcellular fractionation using Opti-prep ultracentrifugation. Each subcellular fraction was analyzed for ACAT1 protein content and for ACAT1 enzyme activity in vitro. The results show that, under normolipidemic condition, the ACAT1 protein is mainly distributed among the middle density fractions characteristic of the ER; the ACAT1 enzyme activity in vitro in each fraction is low. After treating the cells with aggregated LDL, a significant portion of the total ACAT1 protein emerge in a low buoyant density fraction; the ACAT1 enzyme activity in this fraction is much higher than those present in the normal ER fractions. Further purifications by using differential centrifugation and immunoinmunoprecipitation procedures disclosed that the low-density, ACAT1 activity rich fraction possesses markers for both the ER and for the trans-Golgi network, but are devoid of the marker for the plasma membrane. Confocal microscopic analysis suggested that, in cells treated with aggregated LDL, the ACAT1 signal significantly colocalizes with the trans-Golgi network signal. Overall, these data suggest that cholesterol loading of macrophages induces the formation of a novel cellular compartment derived from ER, and is rich in cholesterol content. ACAT1 residing in such compartment can efficiently esterify cholesterol, thus preventing excessive build up of free cholesterol in the ER.

P282 12/15-lipoxygenase Deficiency Decreases Neointimal Formation in Male ApoE-knockout Mice in Response to Endothelial Denudation
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Background: Vascular smooth muscle cell (VSMC) proliferation is a key component of the response to injury in vascular disease. The hyperplastic aipoE deficient mouse (ApoE-/-) is widely used as a model of neointimal formation in response to carotid wire injury. 12/15-lipoxygenase enzyme (12/15-LO), homologous to human 15-lipoxygenase, has been implicated in the pathogenesis of VSMC proliferation in vitro and in response to endothelial denudation in the normopilemic rat carotid endotelial denudation model. The in vivo role of 12/15-LO on response to injury in normolipemic state is unknown. 12/15-lipoxygenase is deficient in both ApoE and 12/15-LO (DK) will have reduced neointimal formation following endothelial denudation as compared to ApoE-/- mice. Materials/Methods: DK and ApoE-/- mice were placed on Western diet at 10–12 weeks of age. After one week, wire endothelial denudation in the normolipemic rat carotid artery (LCA) was performed. Mice were euthanized 28 days following treatment and the LCA harvested. Endothelial denudation was confirmed by immunohistochemistry analysis. Results: Nineteen DK (9 m, 10 f) and 24 ApoE-/- mice (13 m, 11 f) were entered into the study. There was no statistically significant difference between genotypes when comparing cholesterol, triglycereide, LDL and glucose levels. Neointimal area was reduced by 54.6% in DK compared to ApoE-/- mice (264 ± 864 μm² vs. 5893 ± 880 μm², p = 0.033) but not in females (40818 ± 7267 μm² vs. 33479 ± 7176 μm², p = 0.055). Conclusion: Neointimal formation following endothelial denudation is reduced in male DK mice, providing evidence that 12/15-LO is a key regulator of neointimal growth in response to vascular injury in the hyperplastic ApoE-/- mouse. Interestingly, there is no difference in the neointimal response to injury in female DK mice, suggesting a gender specific effect of 12/15-LO.
ACE2 Expression in Adipose Tissue Is Regulated by High-fat Diets but Not by Angiotension II

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Objectives: Angiotension converting enzyme type-2 (ACE2) is a monocarboxypeptidase which cleaves both angiotension (Ang) I and II to produce Ang-17. Recent data suggest that ACE2 functionally controls the renin-angiotension system (RAS), metabolizing AngII and regulating blood pressure. ACE2 mRNA is abundantly expressed in mouse adipose tissue, and increases markedly during differentiation of 3T3-L1 cells. In kidney, heart, and aorta, ACE2 is regulated by AngII, ACE inhibitors or AT1 receptor (AT1R) antagonists. The purpose of this study is to define mechanisms for regulation of adipocyte ACE2, focusing on components of the RAS, or high-fat (HF) feeding. Methods and Results: We examined the effect of AngII, losartan, or an AT2 receptor antagonist on ACE2 mRNA abundance in differentiating 3T3-L1 adipocytes. While ACE2 mRNA increased markedly during adipocyte differentiation, there was no effect of treatments. In adipose tissue from female AT1R deficient mice, ACE2 mRNA abundance was not altered. To determine the effect of high fat (HF) feeding on adipose ACE2 expression, C57BL/6J mice were fed either normal laboratory chow or a high-fat diet with 60% Kcal as fat (acute) (1 week) or chronically (20 weeks). Female AT1R−/− mice were also fed normal or HF diets for 20 weeks. ACE2 mRNA expression and activity in adipose tissue were increased within 1 week of HF feeding in C57BL/6J mice. At 20 weeks, body weight (BW) was increased in mice fed HF diet over normal diet (p<0.05). However, there was no difference in ACE2 mRNA expression between HF and control groups. The level of inflammation is associated with different pathogenic processes and is a novel truncation termed apoB-13.7. The apoB-82 homozygote is asymptomatic despite her extremely low levels of LDL-C (13 mg/dl). Moreover, fat-soluble vitamin levels are normal, possibly due to spared secretion of apoB-48 and increased levels of high-density lipoprotein cholesterol (89 mg/dl). NAFLD was observed in 7 of the 14 subjects, including both patients with apoB-82 and apoB-13.7. The parents of the apoB-82 homozygote, also, had fatty liver. We performed liver biopsy in the apoB-13.7 patient, who was diagnosed as severe fatty liver by computed tomography. The liver showed simple steatosis but no diagnostic evidence of NASH. Conclusions: Our results demonstrate that apoB gene mutations might not be rare and that NAFLD might be frequent in Japanese hypobetalipoproteinemia.

Insulin Inhibits Inflammation in Endothelial Mice in a Phosphatidylinositol 3-Kinase-Dependent Manner

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Abstract Insulin reduces inflammation and morbidity in critically ill patients when used to control pro-inflammatory hyperglycemia. Recent studies indicate that insulin may have glucose-independent anti-inflammatory effects in endotexia models. To date, the mechanism by which insulin reduces inflammation has not been elucidated. Insulin is a well known activator of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway. We hypothesize that insulin-induced activation of the PI3K/Akt pathway mediates its protective effects during endotexia. We have shown that activation of this pathway reduces LPS induction of pro-inflammatory cytokines and mortality in endotexia. The objectives of this study were; 1) to establish a model that tests if insulin decreases inflammation, morbidity, and mortality during endotexia and 2) to determine if these protective effects are mediated by activation of the PI3K/Akt pathway. Using a non-hyperglycemic mouse model of endotexia, we continuously administered a very low dose of insulin that did not alter glucose levels. We found that insulin decreased plasma levels of IL-6, TNF-α, and sICAM-1, and decreased morbidity and mortality. Furthermore, the effects of insulin on pro-inflammatory cytokines and mortality are abolished by the anti-inflammatory effects of insulin. We conclude that insulin reduces LPS-induced inflammation, morbidity, and mortality through activation of the PI3K/Akt pathway. These findings may ultimately have important implications regarding the use of insulin for treating normoglycemic critically ill patients.

An Insertion Mutation in the Promoter Increased Myosin Light Chain Kinase Expression in Hypertension

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Introduction- Blood pressure is a strong genetic trait that is determined by quantitative trait loci of an individual. Many candidate genes responsible for regulating blood pressure have been identified. However, relatively few studies have focused on the genes for smooth muscle contractile proteins despite the fact that smooth muscle contractility and growth are key contributors to vascular function and resistance. Smooth muscle contraction is regulated by the actin-myosin II interaction, which in turn is regulated by phosphorylation and dephosphorylation of regulatory myosin light chain (RLC). The RLC phosphorylation is catalyzed primarily by smooth muscle myosin light chain kinase (smMLCK). Therefore, changes in the expression of smMLCK could elicit changes in blood pressure. Hypothesis- We hypothesized that a genetic mutation in the smMLCK promoter could alter smMLCK expression and contribute to the development of high blood pressure. Methods and Results- We investigated the regulation of smMLCK gene expression using spontaneously hypertensive rats (SHR) as an experimental model. Expression of smMLCK in arteries increases during the development of high blood pressure and is always greater in blood vessels from SHR compared to normotensive rats. Analysis of the DNA sequences of the promoters isolated from SHR and normotensive rats revealed that SHR contain a 12 bp insertion. This insertion consists of 6 pairs of CT repeats and does not alter a-1 DNA structure, an intramolecular triple helix DNA structure in supercoiling DNA. This structure positively regulates the promoter activity in SHR partly by increasing histone dynamics in the region. In vivo, inhibiting smMLCK activity decreases blood pressure in SHR. Conclusion and Prospective- These data provide novel insights into the genetic factors that increase blood pressure and demonstrate the importance of smMLCK expression in the development of hypertension in SHR. These animal studies lay the foundation for studies on human hypertension and possibly provide new insights into diagnosis and treatment of hypertension in humans.

Apolipoprotein B Gene Mutations and Fatty Liver Disease in Japanese Hypobetalipoproteinemia

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Background: Familial hypobetalipoproteinemia (FHBL) is an autosomal dominant hereditary syndrome characterized by decreased plasma levels of low-density lipoprotein cholesterol (LDL-C). FHBL is considered as genetically heterogeneous, and the best-characterized cases are those mutations of the apolipoprotein B (apoB) gene produce truncated apoB. FHBL is thought to be related to nonalcoholic fatty liver disease (NAFLD), which may progress to nonalcoholic steatohepatitis (NASH) or liver cirrhosis. However, few data exist regarding the relationship between FHBL and NAFLD. Methods: To screen truncated apoB variants associated with FHBL, we performed complete 3’ of apoB and 14 exons from 12 14 seed nested multiplex PCR. For those without truncated apoB in plasma, we performed PCR single-strand conformational polymorphism analysis of the apoB gene to detect truncated apoB shorter than apoB-30, which is usually absent from plasma. Results: We identified two individuals with truncated apoB: an apoB-82 homozygote and a heterozygote for apoB-82/13.7. The apoB-82 homozygote is asymptomatic despite her lowered serum HDL-C levels. The apoB-82 heterozygote had lower serum HDL-C levels compared to his siblings. Both patients had normal liver function tests and there was no histological evidence of steatosis or inflammation. Conclusion: These results demonstrate that FHBL is associated with the disease.
reactive protein (hs-CRP), interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α).

Comparisons were made between aortic dissection and the control groups, patients with Marfan syndrome and those without, and patients with and without leakage/rupture. The correlations with the survival outcome were also evaluated. Results: Compared to the control, the patients with aortic dissection had significantly higher hs-CRP (1.98 ± 0.66 vs. 1.07 ±

0.11 mg/dl, p < 0.001) and IL-6 (25.26 ± 16.92 vs. 1.32 ± 0.76, p < 0.001). The level in patients without Marfan syndrome was even higher (hs-CRP: 2.26 ± 2.89 mg/dl, IL-6: 30.40 ± 15.36 pg/ml, p < 0.05 and 0.001 vs. control). For Marfan syndrome, only IL-6 was significantly higher than control (12.23 ± 13.94 pg/ml, p < 0.05) and the extent was smaller. The hs-CRP level was not significantly different from the control. In patients with evidence of leakage/rupture, the IL-6 was higher than those without (33.04 ± 16.02 vs. 21.55 ± 18.55 pg/ml, p < 0.05). No significant differences were noted in IL-1β and TNF-α. For prognosis, the patients who failed to survive to discharge had significantly lower hs-CRP than those who survived (0.72 ± 1.16 vs. 1.32 ± 0.76 mg/dl, p < 0.05). The level was significantly different from the control. Conclusion: Acute aortic dissection is associated with increased IL-6 and IL-6. IL-6 may implicate different pathogenic processes and the chance of leakage/rupture. Poor initial hs-CRP response, on the other hand, is correlated with poor survival prognosis.

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PKD, a Novel Component in Lysophosphatidylcholine-triggered Signaling Pathway, Controls EgR-1 Expression in Monocytic Cells

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Monocyte activation is an important early event in the development of atherosclerosis and other inflammatory diseases. The nature of monocyte activation is not completely understood. We report here that lysophosphatidylcholine (lysPC), a prominent component of oxidized low density lipoprotein and cellular lipid lesions, induces rapid and marked phosphorylation (PKD) activation in monocytic THP-1 cells. Our data also reveal that PKD activation is required for the activation of both ERK and p38 MAPK. Activation of ERK MAPK, but not p38 MAPK, controls the expression of the early response gene (Er), which has been reported to be a key signalling target of PKD activation. Our results have also identified that, of the three components of PKD, namely PDK1, PDK2 and PDK3, only PDK2 is expressed in these cells. PKD2 controls Er-1 expression through ERK MAPK after lysPC activation. Our results reveal that PKD is a novel intracellular component in lysPC-induced mononuclear cell activation. Our findings provide a novel function for PKD in the regulation of Er-1 expression. Using multiple approaches, including sirNA silencing and dominant negative mutants, we conclude that lysPC-induced PDK2 activation is required for Er-1 expression in monocytic THP-1 cells. Our results suggest a role for PKD in the development of atherosclerosis.

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MCP-1/CCL2 Involves AT1 Receptor Antagonism Induced Anti-inflammation via PI3K/Akt in SHR

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To understand what is the mechanism in the ATIA-antia-induced anti-inflammation, we investigate whether the MCP-1 and it’s receptor involved in this process and how to modified the MCP-1/CCL2. We investigated the effect on anti-inflammatory macrophage cell lines and macrophage cell lines, with H2B playing a particularly prominent role. On TG-induced macrophages, H2B contributed ~45–50% of the Pig binding capacity, whereas α-actin, annexin II and p11 contributed ~25%. In an in vitro matrigel invasion assay, a function of H2B in the cellular response could also be demonstrated. Interestingly, treatment of mice with anti-H2B Fab led to a marked reduction in macrophage recruitment (~50%) towards TG, whereas Fab to another Plg-R or nonimmune Fab had limited effects. Taken together, these data suggest that multiple Plg-R contribute to Pig binding to macrophages, and among these, H2B plays a very prominent role.

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Histone H2B as a Functionally Important Plasminogen Receptor on Macrophages

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Active inflammation has been identified as a defining feature of vulnerable atherosclerosis plaques which rupture and lead to arterial thrombosis and cardiovascular events, such as myocardial infarction, stroke and peripheral occlusive disease. Plasminogen (Pig) facilitates macrophage migration to sites of inflammation and injury. The cellular functions of Pig depend upon its binding to receptors (Pig-Rs), which facilitate its activation to plasmin (Pim), protect Pim from inhibition and harness proteolytic activity to the cell surface. However, the particular Pig receptors (Pig-Rs) that are involved in Pig-mediated macrophage migration during inflammation are not well defined. We have investigated the expression of three previously characterized Pig receptors (Pig-α, -β, -γ), α-integrin, and &-integrin in two mouse macrophage cell lines (RAW264.7 and J774A.1) and on thymicoid (TG) induced mouse peritoneal macrophages. In addition, we have also characterized surface expression and function of histone H2B (H2B), a newly identified Pig-R on these cells. Using Fab fragments of anti-H2B, anti-α-integrin, and anti-annexin II mAbs, we found that each specifically blocked binding of their target proteins, we have shown that all of these receptors were contributed to Pig binding to the macrophage cell lines, with H2B playing a particularly prominent role. On TG-induced macrophages, H2B contributed ~45–50% of the Pig binding capacity, whereas α-actin, annexin II and p11 contributed ~25%. In an in vitro matrigel invasion assay, a function of H2B in the cellular response could also be demonstrated. Interestingly, treatment of mice with anti-H2B Fab led to a marked reduction in macrophage recruitment (~50%) towards TG, whereas Fab to another Plg-R or nonimmune Fab had limited effects. Taken together, these data suggest that multiple Plg-R contribute to Pig binding to macrophages, and among these, H2B plays a very prominent role.

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Preferential Accumulation of CX3CR1bright Dendritic-like Cells in Atherosclerotic Mouse Aortas

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Mouse and human blood contain both inflammatory and resident-type monocytes. In this study, we tracked these monocyte subsets using CX3CR1+ GFP knockin mice. In blood, both subsets express equal amounts of CD11b and F4/80. Inflammatory, CX3CR1dim monocytes express more MHC class-1, Ly-6G, Ly-6C, CD204, CD11a, CD14, CD54, and I-Aβ than resident-type, CX3CR1bright, which express more CD80. This expression pattern remained unchanged when CX3CR1+ GFP mice were crossed into the ApoE−/− background, except for a 10-fold decrease in Ly-6c expression in CX3CR1dim cells. GFP+ bone marrow cells from CX3CR1−/− mice express equal amounts of GFP and no CD11c when differentiated into macrophages by M-CSF, but high levels of GFP and CD11c when differentiated to dendritic cells (DCs) by GM-CSF and IL-4. Brightly fluorescent monocytes with a dendritic cell shape were present in the wall of the aorta and the femoral artery of CX3CR1+GFPapoE−/− mice, and their number was tripled by feeding these mice with a western diet. Immunofluorescence showed GFP+ DC-like cells under the endothelial and in the media. Both CX3CR1dim and CX3CR1bright cells in the aortic wall express CD11c and CD86 at similar levels. CX3CR1dim cells express more CD31, CCR2, CD80, F4/80, J4, CD31, CD62L, CD71, and I-Aβ from CX3CR1bright cells, consistent with a more mature of activated DC phenotype. CX3CR1bright cells are at least three fold overrepresented in CX3CR1+GFPapoE−/− mice on western diet, accounting for up to 62% of all GFP+ cells. Adoptively transferred bone marrow cells in atherosclerotic aortas of ApoE−/− recipients were 60% CX3CR1bright after 48h. Our data suggest that CX3CR1bright monocytes preferentially home to atherosclerotic aortas and assume a dendritic cell-like phenotype. Since atherosclerotic aortas also contain large numbers of lymphocytes, it is plausible that these DCs may participate in an active immune response in the vessel wall.

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Development of Bead-based Multiplexed Immunoassays for Simultaneous Quantification of Circulating Cardiovascular Biomarkers in Rat Serum and Plasma

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Rat is one of the common models for biomedical research, including cardiovascular diseases in elucidating mechanism of action, new drug screening and preclinical trials. Levels of soluble biomarkers such as BNP, Troponin T, TIMP-1, sICAM-1, e-Selectin, VEGF, MPO, WVF and Fibrinogen are associated closely with cardiovascular diseases (e.g. atherosclerosis, thrombosis, and hypertension). These are important indicators for disease model establishment, drug in vivo testing and efficacy evaluation. In order to design useful tools for researchers, we developed three multiplexed immunoassay panels based on Luminox™ x MAP technology. Panel A can detect and quantify simultaneously 11 biomarkers (BNP, TIMP-1, MPO, WVF, Troponin T, Troponin I, Ly-6G, IL-6, TNF-α, MCP-1, CCL2, VEGF, PAI-1) from as little as 15ul of samples. Panel B (solute E-Selectin and soluble ICAM-1) and Panel C (Fibrinogen and Adiponectin) use less than 1ul of samples. All three panels require less than 1% cell line in cell culture and less than 1% of peritoneal macrophage. Briefly, diluted samples are incubated overnight at 4 °C in a 96-well microtiter plate with a mixed population of polyethylene beads, which are immobilized with specific capture antibodies. Each bead set contains two internal fluorophores for bead identification. After washing, the captured analytes on beads were incubated at 2 hr at RT with a cocktail of biotinylated detection antibodies. Following subsequent incubation with streptavidin-phycocerythrin, the fluorescent signals on beads are quantified by a Luminox™ Reader. Within each panel, there is no significant cross-reactivity among different antibody pairs. According to individual needs, the panel can be customized to detect select analytes. The assays are analytically robust (sample dilution linearity, 100 ± 25%, spike recovery, 90 ± 20% and reproducible (inter-assay CV%, <10%; inter-assay CV%, <15%). The multiplexed assay panels also may be used for tissue culture supernatant and tissue/cell lysate samples. These new immunoassay panels provide convenient, flexible and economic tools for simultaneously quantifying multiple CVD biomarkers in small volume rat samples.
Cloning of Human Antibodies to Oxidation-Specific Epitopes from Both Innate and Adaptive Immunity

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Oxidation-specific epitopes are present on oxidized LDL (oxLDL), on cells undergoing apoptosis, and in atherosclerotic lesions. Mice have normal germline IgM antibodies (Ab) that recognize oxidation-specific epitopes. These Abs are part of innate immunity and exert important biological functions in atherogenesis such as ability to bind to apoptotic cells, inhibit uptake of oxLDL and apoptotic cells by macrophages, and confer atheroprotection in mice. Ab titers to oxidation-specific epitopes are found in human adult and umbilical cord blood (UCB) plasma and in atherosclerotic lesions, but it is not known if these Abs are germline. Also, the nature and role of Abs in general to oxidation-specific epitopes in human atherosclerosis is poorly understood. Human UCB contains significant IgM Abs to epitopes on oxidation-specific epitopes. These IgM Abs represent a naïve immune repertoire without exposure to exogenous antigens and may thus serve as a general surveillance system to recognize aberrant glycosylation that is similar to that on mouse, have germline Abs to oxidation-specific epitopes that play important roles in atherosclerosis. We have generated human Fab (Ab fragment) phage display libraries from five patients with familial hypercholesterolemia and seven UCB samples and screened these libraries against oxidized LDL to identify Fab clones highly specific to certain oxidation-specific epitopes. Selected Fabs from both the adult and UCB libraries have been shown by deconvolution microscopy and flow cytometry to bind apoptotic cells and also stain atherosclerotic lesions. These results show that both innate and adaptive immunity are involved in the recognition of oxidation-specific epitopes in UCB and these Abs bind to apoptotic cells and atherosclerotic lesions. Further studies may elucidate the role of these Abs in atherosclerosis and provide new insights to diagnosis and treatment of patients.

Comparison of Fenofibrate Alone or Atorvastatin Alone on Plasma Inflammation, Adhesion, and Oxidation Markers in Type 2 Diabetic Subjects with Moderate Hypertiglyceridemia

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Type 2 diabetes mellitus is associated with elevated plasma triglyceride (TG) levels, low HDL-cholesterol (C) and high incidence of cardiovascular disease. HMG-CoA reductase inhibitors or fibrates are frequently used in the treatment of diabetic dyslipidemia but their specific impact on the inflammatory process involved in atherosclerosis remains to be fully characterized. The objective of this two-group parallel study was to investigate the differential effects of atorvastatin 20mg/d alone (n=20) or micronized fenofibrate 200mg/d alone (n=19) on inflammation, adhesion and oxidation markers in type 2 diabetic subjects with moderate hypertension/diabetes. Atorvastatin decreased plasma-C (-38.3%, P<0.0001), plasma-TG (-38.3%, P<0.0001), plasma apo a1 (-44.1%, P<0.0001) and LDL-C (-43.4%, P<0.0001), and increased HDL-C (+16.5%, P=0.007). Atorvastatin decreased plasma levels of CRP (-23.9%, P=0.004), sICAM-1 (-5.4%, P=0.03), sVCAM-1 (-4.4%, P=0.008), sE-selectin (-5.7%, P=0.02), MMP-9 (-39.6%, P=0.04), sPLA2 (-14.8%, P=0.04) and oxLDL (-38.4%, P=0.0001). On the other hand, fenofibrate treatment decreased plasma-C (-12.1%, P=0.0001) and plasma TG (-41.4%, P=0.0002) and increased HDL-C (+15.7%, P=0.04) and HDL-C (-10.5%, P=0.05). Fenofibrate decreased plasma levels of sE-selectin (-6.0%, P=0.04) but increased the plasma levels of sPLA2 (+22.5%, P=0.004). Fenofibrate had no significant effect on CRP levels. In conclusion, the results of the present study suggest that atorvastatin is potent to reduce inflammation, oxidation and monocyte adhesion in type 2 diabetic subjects with moderate hypertriglyceridemia while fenofibrate decreased sE-selectin levels only and had an effect on sPLA2 levels.

Effects of Artemisia princeps Pampanini Cultivated Sajabal on LDL Oxidation and Atherosclerosis in LDLR−/− Mice

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Oxidatively modified LDL (oxLDL) plays a key role in the development of atherosclerosis. Many animal and clinical studies have shown the beneficial results of various antioxidants treatments in reducing atherosclerotic lesions and cardiovascular risks. The objective of this study was to determine the anti-atherosclerotic effects of Artemisia princeps Pampanini cultivated Sajabal. Jacobson isolated from A. princeps Pamc. cv. Sajabal has an LDL-antioxidant activity. The ethanolic extracts of A. princeps Pamc. cv. Sajabal containing 9.7% (w/v) phenolic compounds reduced atherosclerotic lesions in LDLR−/− mice. We assessed the effect of the extracts on the aortic lesion formation and macrophage accumulation at 37% and 43%, respectively, compared to controls. In aorta, the extracts decreased the transcriptional levels of CD36, LOX-1, ABCA1, I CAM-1, VCAM-1, TNF-α, IL-1β, COX-2 and iNOS and increased the levels of CYP7 family. The extracts not only reduced epididymal fat accumulation (28%) and plasma lipid peroxidation (16%), but also corrected associated hyperlipidemia. These findings indicate that the extracts from A. princeps Pamc. cv. Sajabal effectively ameliorates atherosclerotic lesion formation by inhibiting lipid peroxidation and the expression of LOX and CDX-2 and the expression of CDX-2 and the expression of ICAM-1 and VCAM-1 expression, which are crucial for the development of a fatty streak lesion.

Phenotypic Switching in Macrophages by Oxidized Phospholipids Involves Toll-like Receptor 2

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Functional heterogeneity is a hallmark of cells of the mononuclear phagocyte system. Macrophages (Mφs) have been ascribed both pro- and anti-inflammatory properties. Classical activation of Mφs by LPS leads to the production of cytokines such as IL1 and TNF-α, while alternative activation by L4 U12 cause the production of anti-inflammatory cytokines. OxPAPC, a lipoprotein that mimics oxidized phospholipids (oxPLP) that stimulate Mφs towards a unique phenotype, different from the M1 and M2 types. Phenotypic switching of Mφs by OxPLP is regulated by upregulation of KC, Mip2, IL1β, COX-2 and HO-1, while the expression of the typical M1 markers TNFα and IL12, as well as the M2 marker arginase 1 are downregulated. This macrophage phenotype exhibits increased survival, reduced migratory capability and facilitated lipidoprotein uptake, and is present in atherosclerotic lesions. In order to address the question of involved receptors, we used Mφs from mice lacking TLR2. We show that OxPLP-induced expression of the chemokines Mip2 and KC and IL1β and HO-1 is independent of TLR2. To identify molecular structures in OxPLP that determine Mφ polarization by TLR2 we fractionated OxPLP into long chain fatty acids and short chain fatty acids (m/z <782). We could show that TLR2-independent COX-2 is induced by the long chain fraction of OxPLP, while TLR2-dependent KC is induced by the short chain fraction of OxPLP, that contains 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphate (PGP), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphate (PGPC), and Lyso-PC. Together, we show that OxPLP via TLR2 induces a unique M2-phenotype.

Giant-cell Arteritis Is a Not So Rare and Not So Easy to Handle Form of Vasculitis: Analysis of 5 Years of Experience

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Introduction: GCA is a rare systemic vasculitis of uncertain, probably T cell driven immunological origin. Early diagnosis is essential in prevention of increased cardiovascular mortality and impaired quality of life. Objective: Identification of patients affected by GCA and treated in our hospital in the last 5 years. Analysis of their clinical data in order to improve diagnosis and therapy. Method: analysis of clinical history, diagnostic algorithm, efficacy of therapy especially corticosteroid regimen with a follow up period of 5 years. Results: We analysed the data of 24 identified patients (12 men, 12 women, 17 years old to 77 years old), which is a population wider than the expected prevalence. 18 patients showed typical cranial, while 6 patients showed large vessel manifestation which are two distinct entities characterized by different cytokine pattern in the vascular wall. The period of time between the first symptoms and the diagnosis was more than one year that represents the unawareness of this condition. In case of large vessel manifestation vascular ultrasound proved to be essential. Despite of a long steroid therapy (mean 19 months) 2 relapses occurred on the average, which is indicative of the limitation of corticosteroid therapy. Complications resulting from corticosteroid therapy, like diabetes, fracture due to osteoporosis, myopathy, infections were prevalent in the examined population. Conclusion: GCA is probably much more frequent than generally considered. Diagnostic procedures include clinical observation, vascular ultrasound and histology. Corticosteroid therapy is effective to diminish the symptoms, but in the long run it can be regarded suboptimal, therefore new immunological therapy is needed.
severe CAV (p < 0.001). A strong association was found between the need for coronary interventions and the CRP concentration (p = 0.017). Conclusions: The data suggest that a proinflammatory environment rich in CRP favors early development of severe CAV and increases the need for coronary interventions in heart transplant recipients.

Lipoprotein Accumulation and Antigen Presentation in Grossly Normal Human Aorta

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We earlier had found that subendothelial intimacies in grossly normal human aorta express HLA-DR and CD1a molecules that are involved in peptide and lipid/carbohydrate antigen presentation, respectively. We hypothesize that low density lipoprotein (LDL) stimulates antigen-presenting function in subendothelial cells. To investigate LDL distribution in grossly normal subendothelial intima antibodies against apoE were used. Apoptin was visualizing using confocal laser scan microscopy and immunohistochemical methods. We localized apoE both inside the cells and associates with extracellular matrix. Intracellular apoE was found both in CD1a+ and HLA-DR+ cells. CD1a and HLA-DR were expressed not only by typical antigen-presenting monocyte-derived cells but also by resident intimacytes (pericytes and smooth muscle cells). HLA-DR and CD1a molecules were associated with membrane antigen-presenting structures and intracellular structures transporting antigen from endoplasmic reticulum to cell surface through antigen-transporting vesicles. The size of HLA-DR+ and CD1a+ vesicles were similar and ranged from 0.2 to 1 μm. Intracellular apoE was colocalized with CD1a+ vesicles. In HLA-DR+ cells, the majority of vesicles containing apoE were closely associated with HLA-DR+ structures but not colocalized. This suggests the difference in antigen processing occurring in HLA-DR+ and CD1a+ compartments. CD1a+apoE+ cells were stained with association with lymphocytes and macrophages. The hypothesis that increased levels of soluble Gal-3, found in advanced human and murine atherosclerotic plaques of ApoE -/- mice on a high-fat western-type diet showed that Gal-3 expression also increases with age and lesion size (3.1-fold increase from 8 weeks, n = 12) and protein level (2.5-fold), at the mRNA (n = 12) and protein level (n = 9), as determined by qRT-PCR and western blotting analysis. Gene expression analysis of atherosclerotic plaques of ApoE -/- mice on a high-fat western-type diet showed that Gal-3 expression also increases with age and lesion size (3.1-fold increase from 8 weeks, n = 6, to 16 weeks n = 6). In vitro, Gal-3 mediates monocyte chemotraction and causes an up to 10-fold increase in human macrophage expression of pro-inflammatory mediators, such as TNFalpha and RANTES in a dose-dependent manner, as revealed by microarray (Illumina /H11005). Intracellular apoE also increases more than 10-fold during differentiation while variance in apoB mRNA were up-regulated in unstable plaque regions of carotid endarterectomy specimens compared to those of control mice. Our studies suggest that cellular stress and inflammatory lesions. Therefore, it is generally accepted that monocytes and macrophages should play regular roles in the progression of various inflammatory diseases such as infectious diseases, auto-immune diseases and atherosclerosis. In previous our studies, we reported that U937 monocyte-like cells produce C-reactive protein (CRP) by themselves and stimulation with interferon (INF)-gamma could upregulate production of CRP in U937 cells (8th ATVS meeting, 2005). These results revealed that monocytes and macrophages might be one of important resources of CRP in focal inflammatory lesions. Further, we demonstrated that the composition of CRP produced by U937 was changed by stimulation with INF-gamma. This change was enhanced by additional stimulation with oxidized LDL. (IV international symposium on atherosclerosis 2006). However, effects of this CRP is still unknown at all. (Results) U937 cells regularly expressed m-RNA of CRP and the level of m-RNA were increased after stimulation with INF-gamma. Production of CRP has been already observed with Immunocytochemical staining, Westernblotting and Immunoprecipitation. In this study, we examined proinflammatory effects of CRP produced by U937 cells on themselves using the lysate of cells. Cell lystate was prepared by sonication and centrifuge. Cell lystate of U937 cells clearly upregulated expression of inflammation related proteins, such as MCP-1, IL10, COX2, IL-6 and Oxidized LDL receptor. These effects of lysate were enhanced in cells stimulated with INF-gamma with or without oxidized LDL. On contrary, after only CRP was removed by immunoprecipitation, these proinflammatory effects were weakened significantly. (Conclusion) U937 cells are one of precious resources of CRP and stimulation with INF-gamma and LDL can upregulate its production. Co-stimulation with INF-gamma and oxidized LDL are synergistic. These results suggest that monocytes and macrophages in atherosclerotic lesions might accelerate vascular wall inflammation by local production of both native and modified CRP.

A Role for Galectin-3 as an Amplifier of Inflammation in Atherosclerotic Plaque Progression

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Galectins are a family of lectins that are involved in inflammation, cell adhesion, apoptosis and chemotaxis, and can also function as scavenger receptors. We identified Galectin-3 (Gal-3) as a highly abundant transcript in a whole- transcriptome scan of unstable atherosclerotic plaques obtained from carotid endarterectomy specimens to stable regions from the same patient (p < .25 -fold), at the mRNA (n = 12) and protein level (n = 9), as determined by qRT-PCR and western blotting analysis. Gene expression analysis of atherosclerotic plaques of ApoE -/- mice on a high-fat western-type diet showed that Gal-3 expression also increases with age and lesion size (3.1-fold increase from 8 weeks, n = 6, to 16 weeks n = 6). In vitro, Gal-3 mediates monocyte chemotraction and causes an up to 10-fold increase in human macrophage expression of pro-inflammatory mediators, such as TNFalpha and RANTES in a dose-dependent manner, as revealed by microarray (Illumina Sentrix® arrays) and qRT-PCR analysis. Finally, conditioned media from Gal-3 treated human macrophages (after Gal-3 blocking) also mediates chemotraction of monocytes through a pertussis toxin-sensitive, dose-dependent fashion. In conclusion, Gal-3 may represent a new class of inflammatory mediators that contributes to atherosclerotic plaque progression both through monocyte chemotraction and macrophage activation. Gal-3 could serve as a novel therapeutic target in anti-inflammatory strategies for the treatment of atherosclerosis.

P303 Intracellular Stress Activates BZIP Transcription Factors to Induce the Liver Inflammatory Response

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Atherosclerosis, the major cause of heart diseases, is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate and propagate disease lesions. Endoplasmic reticulum (ER) stress is known to induce the unfolded protein response (UPR), an intracellular signaling pathway from the endoplasmic reticulum (ER) to nucleus to protect cells from stress caused by accumulation of unfolded or misfolded proteins. Here we found that ER stress, oxidative stress and pro-inflammatory signaling can interact and merge into each other to activate the liver inflammatory response that contributes to atherosclerosis. XIAP1, ATF6 and CEBB are ER stress-inducible basic leucine Zipper (BZIP) transcription factors of CEBB/ATF family. ER stress and oxidative stress induced by high-homocysteine diet, inflammatory cytokines TNFa, IL6, IL1b or Lipopolysaccharide (LPS) can induce processing of XIAP1, ATF6 and CEBB can interact with each other to activate transcription of the major inflammation genes encoding C-reactive protein (CRP), Serum Amyloid P-component (SAP) and Serum Amyloid A (SAA). In engineered mice defective in ER stress-transducer molecules IRE1a, XBP1, OX2 and/or ATF6, transcription of the CRP, SAP and SAA3 mRNAs and production of secreted CRP, SAP and SAA3 proteins were significantly reduced in response to LPS that induces ER stress, oxidative stress and inflammation, compared to those of control mice. Our studies suggest that cellular stress and inflammatory stimuli activate BZIP transcription factors XIAP1, CEBB and ATF6 to induce the inflammatory response. Those studies provide a molecular basis for which intracellular stress activates liver inflammation, and will be informative to developing novel methods to control inflammatory diseases, particularly atherosclerosis.

P304 Increases in apoB-lipoprotein Secretion During Differentiation of Caco-2 Cells due to Enhanced Transcription of MTP

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Microsomal triglyceride transfer protein (MTP) and apolipoprotein B (apoB) are essential for lipoprotein assembly. In the intestine, the differentiated enterocytes produce apoB-lipoproteins. Caco-2 cells produce apoB-lipoproteins after spontaneously differentiating into enterocyte-like cells in culture. We analyzed changes in apoB secretion and MTP activity in Caco-2 cells cultured on Transwells for 2–3 weeks. ApoB secretion and MTP activity were barely detectable in non-differentiated cells but increased 5 to 6-fold during differentiation. Interestingly, MTP mRNA increased more than 10-fold during differentiation while variance in apoB mRNA were less than 1-fold indicating that induction in MTP, not apoB, is responsible for the increased apoB secretion. We used this model system to investigate the molecular basis for the transcriptional induction of MTP. Studying a series of 5’ end truncated human MTP promoter sequences revealed that a 204-bp sequence, conserved during evolution, was sufficient to simulate differentiation-dependent MTP expression. Site-directed mutagenesis of putative elements in 204-bp and co-transfection of candidate transcription factors revealed that HNF1 and DR1 elements and HNF4a, HNF1a and HNF1b proteins are involved in the basal expression. For differentiation-dependent expression proximal and distal DR1 elements were important. However, differentiation-dependent induction of MTP was not correlated with
changes in HNF1α, HNF1β, and HNF1β. Instead, profiling differentiation-dependent changes in several candidate transcription factors associated with DR1 element unveiled a putative repressor, NRP2-1 (also called EAR or COUP-TF1), whose expression was reduced by more than 50% after Caco-2 cell differentiation. Knockdown of NRP2-1 by siRNA in undifferentiated Caco-2 cells increased MTP promoter activity. In summary, our data indicate increased transcription of MTP enhancer apoB-1000 promoter and increased expression of NRP2-1 during differentiation induces MTP in Caco-2 cells. Tissue-specific regulation of NRP2-1 may provide novel therapeutic solution to control MTP expression in the intestine.

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We previously proposed that the initiation of apolipoprotein (apo) B particle assembly occurs when βV1 domain of apoB folds into a three-sided lipovitamin-like lipid binding cavity to form the apoB “lipid pocket.” We demonstrated, based on experimentally-derived results and molecular modeling, that the N-terminal 1000 amino acid residues (βV1 domain) of apoB (designated apoB:1000) are competent to complete the “lipid pocket” without a structural requirement for the microsomal triglyceride transfer protein (MTP) and that this requirement is fulfilled by a phospholipid-rich particle. These results, however, did not rule out a MTP-mediated lipid transfer to apoB:1000. In this study, we investigated the putative role of MTP in the initial lipidation of apoB:1000 by employing metabolic labeling of stable transformants of McA-RH777 cells with [3H]oleic acid, and [3H]cholesterol in the presence or absence of BMS-179763 and BMS-200150, two inhibitors of MTP lipid transfer activity. BMS-179763 at 0.1 μM and BMS-200150 at 5.10 and 20 μM had no detectable effect on the synthesis, lipidation, and secretion of apoB:1000-containing particles. At 40 μM BMS-200150, the content of intact apoB:1000 production was reduced by 15-20% and the reduced particle size was attributable to the effect of high concentration of this compound generally on hepatic protein synthesis, as reflected in 20-30% inhibition in albumin secretion. In addition, MTP inhibitors had no effect on the lipid composition of secreted apoB:1000-containing particles. Under these experimental conditions, the synthesis, lipidation, and secretion of apoB:1000-containing particles in HepG2 cells were inhibited by 90-97% and secreted particles had a lower content of triglycerides and a higher level of phospholipids. In conclusion, our studies provide compelling evidence that the initial addition of phospholipids to apoB:1000 and the initiation of apoB lipidoprotein assembly occur independently of MTP lipid transfer activity. We propose that a lipid transfer protein other than MTP mediates the formation of the phospholipid-rich primordial apoB particle.

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HDL is a heterogeneous mixture of lipoproteins with a density of 1.063–1.21 g/ml. A powerful approach to address these issues. HEK293 cells were stably transfected with an expression construct that contains cDNA for human apoA-I/clusterin. We used western blots to identify apoJ/clusterin as a protein that is prominent in the culture supernatant. In a tritiated thymidine uptake assay with A7r5 cells, we used this response as a positive control for responsiveness of the A7r5 cells. We know that apoA-I/clusterin possesses heparin binding domains and we propose to utilize affinity chromatography to purify apoJ/clusterin from the culture supernatant. We anticipate that this in vitro approach will provide a consistent source of apoJ/clusterin that is biologically active. The potential is present to determine on vascular smooth muscle cells not only the receptor(s) of apoJ/clusterin in vascular biology.

Diet Enriched with Oxidized Fatty Acids Lowers Triglyceride Levels in Mice

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We have previously shown through cell culture and animal studies that the intestine efficiently absorbs oxidized linoleic acid and atherosclerosis is increased in animals fed a high cholesterol diet in the presence of oxidized linoleic acid. Animals fed non-atherogenic diet did not develop atherosclerosis even in the presence of oxidized fatty acids. Oxidized fatty acids have also been shown to be ligands for PPARs and could induce genes for antioxidant enzymes such as catalase. In the present study, we fed C57BL/6 mice normal mouse diet in the presence of oleic or oxidized linoleic acid (13-oxidohexadecadienoic acid, 13-HODE) at 50 mg per animal per day for 2 weeks. There was no major changes in the lipid or lipoprotein classes in 13-HODE fed animals as compared to oleic acid fed animals, except that the triglyceride values were significantly lower (40.37% of controls) in 13-HODE treated animals (Table). Paraoxonase (PON1) activity did not change between the two groups. We observed a lowering of plasma triglycerides in human subjects who consumed oxidized fatty acids as compared to linoleic acid. The result suggests that oxidized fatty acids may also have anti-atherogenic properties in the absence of dietary cholesterol. However, 13-HODE also appeared to have pro-inflammatory properties which might counteract any potentially beneficial effects. It is possible that 13-HODE might affect the synthesis and metabolism of triglycerides or triglyceride containing lipoproteins. Further studies are in progress.

Native 2D Gel Analysis of HDL Subpopulations: A Proteomics Approach

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HDL is a heterogeneous mixture of lipoproteins with a density of 1.063–1.21 g/ml. A powerful approach to address these issues. HEK293 cells were stably transfected with an expression construct that contains cDNA for human apoA-I/clusterin. We used western blots to identify apoJ/clusterin as a protein that is prominent in the culture supernatant. In a tritiated thymidine uptake assay with A7r5 cells, we used this response as a positive control for responsiveness of the A7r5 cells. We know that apoA-I/clusterin possesses heparin binding domains and we propose to utilize affinity chromatography to purify apoJ/clusterin from the culture supernatant. We anticipate that this in vitro approach will provide a consistent source of apoJ/clusterin that is biologically active. The potential is present to determine on vascular smooth muscle cells not only the receptor(s) of apoJ/clusterin in vascular biology.

In Vitro Production of Active ApoJ/Clusterin

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The traditional source for human apoJ/clusterin is pooled human plasma. The purification protocol is tedious, potentially hazardous, and prone to bacterial contamination. Moreover, there is no potential to produce a modified form of apoJ/clusterin. We utilized an in vitro approach to address these issues. HEK293 cells were stably transfected with an expression construct that contains cDNA for human apoJ/clusterin. We used western blots to identify apoJ/clusterin as a protein that is prominent in the culture supernatant. In a tritiated thymidine uptake assay with A7r5 cells, the same culture supernatant significantly suppresses growth factor stimulated uptake of triglycidyl thiourea in A7r5 cells, as expected. Exogenous apoJ/clusterin evokes a similar suppressive effect on triglycidyl uptake in growth factor stimulated A7r5 cells and we used this response as a positive control for responsiveness of the A7r5 cells. We know that apoJ/clusterin possesses heparin binding domains and we propose to utilize affinity chromatography to purify apoJ/clusterin from the culture supernatant. We anticipate that this in vitro approach will provide a consistent source of apoJ/clusterin that is biologically active. The potential is present to determine on vascular smooth muscle cells not only the receptor(s) but also the domains of the apoJ/clusterin molecule that evoke the protective function of apoJ/clusterin in vascular biology.

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Initiation of Apolipoprotein B-Containing Lipoprotein Assembly Is Independent of Microsomal Triglyceride Transfer Protein Activity

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The human hepatoma cell line, HepG2, secretes apoA-I as free and lipidated (HDL) species. Apo A-I is lipidated intracellularly (20%) and in 1-hour media (=30%; Choshom et al. 2002). The corresponding biogenic itineraries of apos A-I and E3, cysteine-containing apoproteins that dimerize with other cysteine-containing proteins, are less clear. ApoA-I in human plasma and in HepG2 media is homodimeric; plasma apo E3 occurs as 45% monomer, 26% homodimer, and 29% A-E heterodimer. Given that lipid surfaces catalyze dimerization of human apo A-II (Gillard et al 2005), we hypothesized that intracellular lipidation of apo A-II promotes dimerization, and because of its higher lipophilicity, intracellular and newly secreted apo A-I, and perhaps apo E, would be more heavily lipidated than apo A-I. We compared the lipidation of intracellular and newly secreted apoproteins, A-I, E, and A-I. According to density gradient centrifugation and size exclusion chromatography (SEC) apop A-I and E are lipidated intracellularly. Apos A-I and E have distinctive dimerization kinectics: apo A-I dimerizes intracellularly; secreted apo E is monomeric but dimerizes in media. Western blots of SEC fractions revealed that apo occur with LDL, HDL, and as lipid-poor and lipid-free proteins; ~25% of intracellular apo A-I co-elutes with nascent HDL; ~80% of intracellular apo A-I is in the nascent HLD fraction; ~90% of intracellular apo E co-elutes as VLDL. In 2 hour HepG2 media, ~50% of apo A-I is in an HDL fraction; the rest is lipid-poor or lipid-free. Most apo A-I is in the nascent HDL fraction; while most of the apo E is in larger HDL particles as well as on LDL particles. Newly secreted apo A-I is ~100% homodimeric, while apo E is ~100% monomer, but forms homodimers and apo A-E heterodimers over 24 hr. Conclusion: Apo A-I, A-E and E are processed differently in HepG2 cells with respect to lipidation and dimerization, and heterodimerization of apop A-I and E requires association of apo on a common particle.
Delayed in Vivo Catabolism of Small Dense LDL: A Stable Isotope Study

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Background and aims: Small dense LDL (sdLDL) is an emerging risk factor for coronary heart disease. However, detailed in vivo metabolism of sdLDL has been poorly understood. In order to assess sdLDL metabolism, we performed in vivo kinetic studies utilizing stable isotopically labeled leucine in 10 hypercholesterolemic patients and 5 healthy controls. Effects of statin on sdLDL metabolism were also investigated. Methods: Deuterated leucine was injected and blood samples were collected up to 48 hours. sdLDL was isolated by heparin-magnesium (LRK 44:2193, 2003), followed by ultracentrifugation (d~ 1.019 - 1.063 g/mL). VLDL, IDL, and LDL were also isolated by sequential ultracentrifugation. ApoB was precipitated by isopropanol method, then hydrolyzed and derivatized to determine tracer/tracer ratio of apoB by gas-chromatography mass spectrometry. Results: Fractional catabolic rate (FCR) was estimated by SAAMII software. The FCR of sdLDL apoB was 0.23 ± 0.06 pools/day, which was 35% lower than that of LDL apoB of 0.35 ± 0.12 pools/day (p = 0.001). Furthermore, statin therapy significantly increased sdLDL apoB FCR by 67% (p < 0.01), in addition to 94% increase in LDL apoB FCR. Conclusions: This is the first in vivo kinetic evidence of the delayed catabolism of sdLDL which was improved by statin therapy. Therefore, the impaired catabolism is translated into the longer residence time, thus supporting the proatherogenic nature of sdLDL.

IRE1α Restricts Chylomicron Production B by Selectively Degrading MTP mRNA


Microsomal triglyceride transfer protein (MTP) is obligatory for the production of intestinal chylomicrons to absorb dietary fat and fat-soluble vitamins as well as dietary and biliary cholesterol. Inositol-requiring enzyme 1 (IRE1α), a membrane-anchored kinase/ribonuclease, plays a key role in relieving the stress induced by the build-up of misfolded proteins in the endoplasmic reticulum. Although seemingly unrelated, we provide evidence for a cross talk between these two processes to avoid hyperlipidemia during postprandial state. IRE1α is expressed ubiquitously, but the intestinal epithelium expresses an additional protein IRE1β. Plasma cholesterol and triglyceride were significantly increased in cholesterol-fed IRE1α−/− mice due to increased chylomicron production. Moreover, the activity and mRNA of MTP were markedly enhanced in the intestine, but not the liver, of these mice. In human hepatoma Huh7 cells, cholesterol enhanced MTP levels but not when IRE1β was expressed. IRE1β specifically decreased MTP mRNA in these cells. IRE1α had no effect on the MTP promoter, but reduced MTP mRNA levels expressed under a heterologous promoter indicating for post-transcriptional degradation. In IRE1β expressing cells, the degradation of the 3’ end of the MTP mRNA was prevented by siRNAs targeting 5’ to 3’ exonicucleases, XR宁1 and XR宁2. These studies indicate that IRE1β regulates MTP mRNA levels involving post-transcriptional degradation. Thus, IRE1β deficiency leads to intestine-specific induction of MTP and increased lipid absorption in response to a high-cholesterol diet. These findings have potentially important mechanistic and therapeutic implications related to the role of ER stress in the production of intestinally derived atherogenic lipoproteins.

ACAT2 and Human Hepatic Cholesterol Metabolism: Identification of Important Gender-related Differences

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Objective ACAT2 is specifically expressed in hepatocytes and plays a role in hepatic cholesterol esterification in human. To further elucidate its physiologic role in human cholesterol metabolism, liver biopsies from 19 gallstone patients (female/male, 10/9) and 12 gallstone-free patients (female/male, 8/4) were collected, and analyzed for ACAT2 activity and expression. Results Age and BMI were matched between groups and genders, as well as plasma total cholesterol and triglycerides. As expected, HDL cholesterol and Apo AI were significantly higher in females than in males. Hepatic ACAT2 activity did not differ between gallstone and gallstone-free patients and no correlation was observed between the hepatic ACAT2 activity and the biliary cholesterol content. Interestingly, in females, hepatic ACAT2 activity was 1/4 of what observed in males (7.2 ± 1.2 vs. 29 ± 9.1 pmol/min/mg protein, P = 0.01). This gender-related difference was also seen at protein level, but was not found at mRNA level. Furthermore, the hepatic activity of ACAT2 correlated negatively with serum HDL cholesterol (r = 0.48, P < 0.05) and with Apo AI (r = 0.61, P = 0.05). Conclusion A strong gender-related different in hepatic ACAT2 activity is present in human liver, and alterations in hepatic ACAT2 activity seems not to underlay the genesis of gallstone disease. Furthermore, a new role for ACAT2 in the regulation of HLD cholesterol levels may be hypothesized: when hepatic ACAT2 activity is low, free cholesterol may be preferably secreted into HDL particles rather than into bile.

Human Macrophage ABCG1 Expression and Function Is Decreased in Patients with Type 2 Diabetes

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Coronary artery disease is the most common cause of death for people with Type 2 diabetes, with diabetic patients being up to four times more likely to develop coronary artery disease than their non-diabetic counterparts. A key early event in the development of atherosclerosis is macrophage foam cell formation. The ABC transporter, ABCG1, plays an important role in macrophage reverse cholesterol transport. Previous work by our lab in mouse models of Type 2 diabetes show a decrease in macrophage ABCG1 protein expression, leading to a decrease in cholesterol efflux to HDL and increased macrophage cholesterol accumulation. Here, we show similar data in human patients with Type 2 diabetes. Human blood was obtained from consenting patients with and without Type 2 diabetes. Monocytes were isolated and differentiated into macrophages in vitro. Western blot analysis of the human macrophages revealed that ABCG1 protein expression was decreased by approximately 40% in diabetic patients compared to controls, without changes in ABCA1 protein levels. Cholesterol efflux experiments revealed a 30% decrease in cholesterol efflux to HDL by diabetic macrophages compared to controls. Further, we saw no change in efflux to lipid free Apo-A1 by diabetic macrophages, indicating no loss of function of ABCA1. Additionally, macrophages from diabetic patients had significantly more lipid accumulation than non-diabetic patients when challenged with oxidized LDL, as measured by oil red o staining and gas-chromatography. In conclusion, Type 2 diabetes appears to decrease human macrophage ABCG1 expression, resulting in decreased cholesterol efflux to HDL, accumulation of cholesterol esters in macrophages, and increased lipid accumulation. Thus, strategies to upregulate ABCG1 expression in macrophages may be important for preventing atherosclerosis development in diabetes.

The Effect of Simvastatin on Surrogate Markers of Vascular Health in Youth

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Failure to diagnose preclinical CVD in youth misses a major opportunity to prevent the long-term consequences of this disease. We have conducted a pilot study to evaluate surrogate vascular markers (SVMs) that are associated with early arterial injury including flow-mediated vasodilatation, carotid intima media thickness, arterial stiffness, and biomarkers including cell adhesion molecules (ICAM-1 and VCAM-1), myeloperoxidases (asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA)), and C-reactive protein (C-PR). We hypothesized that one or more of these SVMs which are linked to early pathological vascular changes will identify high-risk youth with early vascular injury compared to a healthy group and that these markers will tend to normalize with risk factor reduction. We further hypothesized that one or more of the markers will correlate with the Pathological Determinants of Atherosclerosis in Youth (PDAY) risk score. Ten subjects without any known risk factors and 22 hypercholesterolemic (HC) youth aged 10 –20 yrs. were recruited from the pediatric clinic. The majority of the HC group were from the Pathological Determinants of Atherosclerosis in Youth (PDAY) risk score. Ten subjects without any known risk factors and 22 hypercholesterolemic (HC) youth, aged 10 ~20 yrs. were recruited from the pediatric clinic. The majority of the HC group was also obese. The HC group was randomized to diet + 20 mg of simvastatin vs. placebo for 24 weeks followed by a forced titration to 40 mg vs. placebo for 24 weeks ending with a final evaluation after a 12 week washout period of diet alone. The markers that best distinguished the HC from the control subjects were C-PR (p 0.03), VCAM-1 (p 0.05) and SDMA (p 0.04). With the exception of ICAM-1 and C-PR, the marker values improved in the treatment group more than the placebo group and the relative changes were the largest for the methylarginines – a marker closely tied to insulin resistance. The markers that demonstrated the highest correlation with the number of risk factors were VCAM-1 (r 0.45, p=0.05) and SDMA (r 0.48, p=0.04). PDAY risk scores were calculated for each subject and SDMA (p=0.50, p=0.03) and ApoSDMA (r =0.50, p=0.04) again emerged as the most highly correlated SVMs. Consistent with the relatively low PDAY risk scores and a minimally thickened IMT, the data suggest that the majority of subjects do not have advanced atherosclerotic changes. We conclude that SVMs are a useful index of vascular injury in high-risk youth.

Dietary Cholesterol-Mediated Stimulation of Chronic LXRα Activation, Dyslipidemia, Obesity, and Insulin-resistant Diabetes: Evidence for Synergistic Interactions with Dietary Fat and Fructose

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Evidence is presented for an important link between dietary cholesterol, chronic LXRα activation, and development of severe dyslipidemia and insulin resistance. Previously, our
Improves dyslipidemia, insulin resistance and tissue TG content in high-fat-fed Idd4 (-/-) mice, suggesting a novel approach for treatment of the metabolic syndrome.

Vascular Function of LOX-1 Overexpressing Mice on High-fat Diet

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Background: The major receptor for endothelial uptake of oxidized low-density lipoprotein (oxLDL) is lectin-like oxLDL receptor-1 (LOX-1). In this study, we analyzed the impact of high-fat diet on vascular function in transgenic mice overexpressing LOX-1. Methods and Results: In LOX-1 overexpressing mice, bovine LOX-1 transgene was mapped by fluorescence in situ hybridization to chromosome 3 (region 3q34-8) of the mouse genome and found to be inserted in multiple copies. Parallel feeding studies performed onagematched rats with high fructose and high fat diets supplemented with either varying amounts of cholesterol (0.05% or 0.25%) or the specific LXR agonist T0901317 (25 mg/kg) for 14 days. Interestingly, the lipid and lipoprotein profile of hamsters fed diets supplemented with high cholesterol (0.25%) were closely similar to those in hamsters treated chronically (2 weeks) with an LXR agonist, indicating that LXR activation may be at least partially responsible for the chronic effects of cholesterol. The data clearly implicates dietary cholesterol, synergistically acting with dietary fat and fructose, as a major determinant of the severity of insulin resistance and dyslipidemia, an affect possibly mediated via chronic LOX-1 activation.

The concentration response curves of the NO donor nitroprusside did not differ between LOX-1- and WT mice. The membrane potential in the saphenous artery was -75.8 ± 1.1 mV in LDLR-/- mice and -73.7 ± 0.5 mV in WT. Conclusion: We conclude that LOX-1 receptor overexpression on high-fat diet can affect vascular function.

The Citrus Flavonoid Naringenin Inhibits Apob100 Secretion and Improves Dyslipidemia and Insulin Resistance in High-fat-Fed LDLR-/- Mice

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Objective: Periodontal disease is a bacterial infection, which has been classified as a local chronic inflammation. The literature suggested that a link exists between the presence of severe periodontitis and several systematic health changes including an altered lipid metabolism. The aim of this study was to investigate the relationship between chronic periodontitis and serum lipid levels. Material and Methods: The level of serum lipids(DLGL, TG, HDL and LDL) of total 30 patients with Chronic periodontitis (CPTN) score II & IV ranging in age between 30 to 40 years were examined and compared with data obtained from 30 healthy individuals(control group). The relationship of serum lipids and periodontal disease and CPTN index was tested by means of SPSS software (Ver 13.0). Results: The present of periodontal disease was significantly related with higher total cholesterol in case group (P<0.05). Triglycerides and HDL were higher in patients but no statistically significant differences were observed with control group. LDL did not show any difference between case and control groups. Conclusion: Analysis of these data revealed that Chronic periodontitis enhances the changes in occurrence of Hyperlipidemia in healthy people. The findings of this research support the reports linking increased prevalence of changing serum lipids among patients with periodontal disease.

Lipoprotein Subtraction Procedures Differentially Predict Changes in Lipoprotein-associated Phospholipase A2 During Prescription Omega-3 Therapy

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Lipoprotein-associated phospholipase A2 (LP-PLA2), a secretory product of macrophages, circulates bound to low- (LDL) and high-density lipoprotein (HDL) particles, and is an independent predictor of cardiovascular event risk. The present investigation assessed the influence of triglyceride lowering with prescription omega-3 acid ethyl esters (P-OM3) on LP-PLA2 concentration and lipoprotein subtraction levels (assessed by nuclear magnetic resonance spectroscopy) in men and women with triglycerides 200 to 499 mg/dl while on statin therapy. After 8 weeks on simvastatin 40 mg, 256 subjects were randomly assigned to 4 groups: Placebo (P), P-OM3 (0.4 g/kg per day), or a combination of these agents, administered for 8 weeks. P-OM3 treatment lowered LP-PLA2 concentration (231 to 200 ng/ml; p=0.002 vs placebo). The LP-PLA2 response was not significantly related to changes in LDL or HDL cholesterol levels, but was associated with changes in LDL (r=0.30, p=0.002) and HDL (r=0.25, p=0.01) particle concentration (r=0.18, p=0.05). The P-OM3 concentration (r=0.20, p=0.01) particle concentration was not associated with the LP-PLA2 response. Changes in medium (r=0.24, p=0.02) and large (r=0.25, p=0.01) HDL particle concentrations were both associated with the LP-PLA2 response, but in opposite directions. Thus, P-OM3-induced carbon tetrachloride (CCl4) interferes with triglyceride secretion by unknown mechanisms and causes steatosis. CCl4 decreased plasma lipids, apolipoproteins, MTP activity and protein, and increased intestinal as well as hepatic lipids in a combination and time dependent manner. CCl4 decreased apoB-lipoprotein production, MTP activity and protein, had no effect on MTP mRNA, and increased cellular retention of triglycerides in primary enterocytes, colon carcinoma and hepatoma cells. Metabolic labeling revealed that CCl4 had no effect on MTP synthesis, but induced its post-translational degradation involving ubiquitylation and proteasomal degradation. In contrast, a MTP-antagonist inhibited lip transfer activity without reducing protein levels. We hypothesized that CCl4 might covalently modify MTP and signal for its degradation. Incubation of cells with 14CCCl4 resulted in its incorporation into MTP and the amounts of modified MTP increased when proteasomal degradation was inhibited. In contrast, inhibition of proteasome function increased MTP levels. These studies indicate that CCl4 as a major target of CCl4 and its degradation is a novel mechanism involved in the onset of steatosis, and suggest that inhibition of protein synthesis may prevent some forms of steatosis.

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changes in Lp-PLA2 are associated with changes in LDL and HDL particle concentrations, and differentially related to changes in subfractions of these lipoproteins. These findings are consistent with those of prior studies showing that Lp-PLA2 is enriched in small, dense, electrophoretic LDL particles and may have implications for understanding how lipid therapies influence Lp-PLA2 and associated cardiovascular risk. Funding provided by Reliant Pharmaceuticals.

**P329**

The Lipid Peroxidation Product Malondialdehyde Impairs ABCA1 Cholesterol Export from Cells Through Site-specific Crosslinking of Apolipoprotein A-I

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Objective - LDL extensively modified by malondialdehyde (MDA) is a classic ligand for scavenger receptors that promote macrophage foam cell formation in vitro. Protein-bound MDA has been detected in atherosclerotic lesions by immunohistochemistry, implicating lipid peroxidation in the pathogenesis of atherosclerosis. Previous studies have demonstrated lower atherogenicity of MDA-modified LDL in vivo. To date, MDA has focused on LDL and relied on antibodies that react with unknown epitopes. Remarkably little is known about the specific sites and chemical nature of MDA adducts in lipoproteins. Moreover, the possibility that MDA-modified HDL plays a role in atherogenesis has received little attention. We have investigated the possibility that one important target for MDA might be apolipoprotein A-I (apoA-I), the major HDL protein. Results - Lipid-poor apoA-I promotes efflux of cellular cholesterol from macrophage foam cells by the ABCA1 pathway. ApoA-I exposed to increasing concentrations of MDA progressively and dramatically lost its ability to remove cholesterol from cultured VLDL. Using mass spectrometry, we demonstrated that specific lysine residues (K118, K133, K195, and K226) as well as the N-terminal amino group were modified by MDA in high yield. Importantly, cross-linked lysine residues were identified as the major product of apoA-I exposed to MDA. We observed significantly faster movement on non-denaturing PAGE of MDA-modified apoA-I, suggesting that cross-linking between lysine residues condenses the molecular structure of the apolipoprotein. One major cross-linking site in apoA-I involved K226, which is located in helix 10 of the protein. This region plays a critical role in triggering apoA-I lipid association and sterol efflux by the ABCA1 pathway. Conclusions - These observations suggest that the high reactivity of MDA with this site's secondary structure residues. The work presented in this abstract highlights the significance of understanding the specific sites and chemical nature of MDA adducts in lipoproteins by using mass spectrometry.

**P330**

VLDL from Obese Zucker Rats Contain Elevated Eicosanoids and Octadecanoids (Oxylipins) That Are Released by Lipoprotein Lipase

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Objective: Oxidized fatty acid metabolites (oxylipins) such as eicosanoids and octadecanoids are produced in response to inflammation and exposure to oxidized lipids. Oxidized fatty acids such as malondialdehyde (MDA) are elevated in atherosclerotic lesions and contribute to atherogenesis by impairing cholesterol removal from artery wall cells. ABCA1. Our observations indicate that MDA may interfere with normal HDL function by promoting efflux of cellular cholesterol from macrophage foam cells by the ABCA1 pathway.

**P331**

A Novel Role for Apolipoprotein A-V: Association with Intracellular Lipid Droplets

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Accumulating evidence indicates apolipoprotein A-V (apoA-V) is an important regulator of triglyceride (TG) metabolism. Studies with mice and human show that a deficiency of apoA-V is associated with increases in plasma TG levels. Given that plasma levels of apoA-V are extremely low, we hypothesized that this protein functions intracellularly by affecting the assembly and/or secretion of apoB-containing particles. Overexpression of apoA-V in Hep3B cells cultured in medium supplemented with oleic acid altered neither the amount of apoB secreted nor the density distribution of apoB-containing particles. Fluorescence microscopy studies were carried out on oleic acid supplemented McARae-RH777 cells expressing human apoB100 and apoA-V to determine whether these proteins traffic together in the secretory pathway. Confocal fluorescence microscopy images revealed that apoA-V does not interact with apoB intracellularly. Whereas apoB localized to the endoplasmic reticulum, apoA-V was found in a distinct cellular compartment comprised of a cluster of spherical structures. Nile Red fluorescence staining identified these structures as intracellular lipid droplets. ApoA-V, green fluorescent protein fusion protein localized to the surface of the lipid droplets and co-localized with adipophilin, a known hepatocyte lipid droplet surface protein. The data reveal a unique association of apoA-V with intracellular neutral lipid droplets suggesting a function for this protein in lipid storage and/or mobilization.

**P332**

The Influence of Apolipoprotein A-I Introduction on Apolipoprotein A-I Conformation in High-density Lipoproteins

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The metabolism of high density lipoprotein (HDL) is likely influenced by the interaction and conformation of its two major protein constituents; apolipoprotein (apo) A-I and apoA-II. To understand the impact of apo A-I on apoA-I in HDL we have begun to monitor apoA-I conformational changes on mixed apoA-I/A-II discoidal HDL particles (LpA-I/A-II) compared to apoA-I only HDL particles (LpA-II). Homogeneous, 16 LpA-I/A-II was reconstituted with apoA-I. apoA-I in a 2:1 ratio and a total of three protein molecules per particle. These particles were subjected to analysis by a cross-linking and mass spectrometry approach previously used to study LpA-I and discoidal apoA-II HDL (LpA-II) particles. After cross-linking, delipidation and exhaustive tryptic digestion of LpA-I/A-II were specifically searched for the short and long range apoA-I cross-links we previously identified in LpA-I particles. Almost all short-range cross-links (7/8) previously identified in LpA-I particles were present in the LpA-I/A-II particles. However, only 2/9 long range cross-links were present. Furthermore, all short range cross-links (5/5) pertinent to LpA-II were present in Lp-A-II but none of the long range cross-links are found (8/8). These observations strongly suggest that, when present together in a single HDL particle, both apoA-I and apoA-II adopt a distinct conformational conformation in the cross-linking experiments. These results were obtained using LpA-I/A-II particles subjected to a high pressure homogenization with no detergent followed by amino propranolol. The conformational differences between HDLs in vivo may be due to high shear stress in vivo.

**P333**

Helix 10 Mutations in ApoA-I from Atherosclerosis-sensitive Mouse Result in Decreased Lipid Binding and Cholesterol Efflux from Macrophages

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The C57BL/6J (C57) mouse strain exhibits atherosclerotic lesions that are at least five-fold larger than in other strains of mice and has significantly lower plasma cholesterol levels compared to apoA-I, the main structural protein found in HDL. To determine the specific sites and chemical nature of MDA adducts in lipoproteins by using mass spectrometry.

**P334**

Reduced Plasma Corticosterone Levels in LDLr-/-, ApoA-I-/- Mice: A Model to Study the Role of the HPA Axis in the Metabolic Syndrome

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ApoA-I, the main structural protein found in HDL delivers the majority of cholesterol required for interstitial lipid storage and for lipid droplets associated with peripheral fat tissue. Our previous studies have been shown to have 10-fold less adrenal cholesterol compared to LDLr-/- mice. This decrease in adrenal cholesterol is mainly due to the reduction in adrenal esterified cholesterol levels. The goal of this study was to determine if the reduction in adrenal cholesterol ester would lead to changes in adrenal function and alter plasma corticosterone and ACTH concentrations. Both LDLr-/- and LDLr-/- mice fed chow or an atherogenic diet for 8 weeks were subjected to analysis by a cross-linking and mass spectrometry approach previously used to study LpA-I and discoidal apoA-II HDL (LpA-II) particles. After cross-linking, delipidation and exhaustive tryptic digestion of LpA-I/A-II were specifically searched for the short and long range apoA-I cross-links we previously identified in LpA-I particles. Almost all short-range cross-links (7/8) previously identified in LpA-I particles were present in the LpA-I/A-II particles. However, only 2/9 long range cross-links were present. Furthermore, all short range cross-links (5/5) pertinent to LpA-II were present in Lp-A-II but none of the long range cross-links are found (8/8). These observations strongly suggest that, when present together in a single HDL particle, both apoA-I and apoA-II adopt a distinct conformational conformation in the cross-linking experiments. These results were obtained using LpA-I/A-II particles subjected to a high pressure homogenization with no detergent followed by amino propranolol. The conformational differences between HDLs in vivo may be due to high shear stress in vivo.
Profound Cardiac Metabolic Changes Caused by Coronary Disease

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We have recently shown that apolipoprotein E knockout (apoE–/–) mice fed a high-fat, Western-style diet for 6 months, develop occlusive coronary lesions and show evidence of myocardial infarction. The same mice fed a normal rodent diet appear to remain healthy, and therefore provide excellent in vivo controls for the study of cardiovascular disease. Thus, the aim of this work was to investigate the direct myocardial effects of coronary disease. Male apoE–/– mice were weaned onto a normal rodent diet, and at approximately 8 weeks old, animals were either switched onto a high-fat, Western-type diet (21% fat; 0.15% cholesterol) or were maintained on the normal diet for 6 months. Islands were used to measure cardiac metabolites and function. Isolated myocytes were used to measure mitochondrial flux (NAD+/NADH ratio), contractile function and calcium transients. Mice fed high-fat diet had significantly increased levels of cardiac lactate (from 42 ± 6 to 63 ± 9 mmol/g protein), decreased glycogen content (from 0.077 ± 0.008 to 0.036 ± 0.013 mg/g wet weight) and decreased levels of ATP (from 16 ± 0.9 to 11 ± 1 mmol/g protein). Evidence of metabolic stress in diseased hearts was confirmed in isolated perfused myocytes which showed increased NAD+/NADH ratio (from 0.27 ± 0.02 to 0.35 ± 0.02). These metabolic differences did not alter functional characteristics of isolated perfused hearts or myocytes. However, both isolated myocytes and intact tissues from diseased hearts with penetrated disease were significantly more resistant to cardiac insults than control animals. In conclusion, coronary disease induced by high-fat diet has profound metabolic effects on the myocardium. These changes appear to alter vulnerability to cardiac insults, which may be due to ischemic preconditioning.

Prediction of the Localization of High-Risk Coronary Atherosclerotic Plaques Based on Low Endothelial Shear Stress: A Serial IVUS and Histopathology Natural History Study


Background: The role of low ESS in the progression of atherosclerotic plaques and evolution of these plaques to thin cap fibroatheromas (TCFA) has not been studied. We investigated the effect of low ESS in the development of TCFA in swine coronary arteries. Methods: In 11 diabetic hyperlipidemic swine, IVUS-based 3D reconstruction of the coronary arteries was performed at baseline (wk 23) and follow up (wk 30). Baseline ESS was calculated using computational fluid dynamics, and plaque-free segments of interest of 3 mm length were identified (n=142). Coronary arteries (n=31) were harvested at follow up, cryosectioned at the subsegments of interest and stained histologically. Intima/media ratio, min cap thickness, lipid deposition and inflammation were quantified and atherosclerotic lesions were histopathologically classified to minimal (MIN), intermediate (INT) and TCFA. Results: The magnitude of low ESS was significantly associated with larger plaque size, increased lipid deposition, inflammation and cap thinning (P<0.001). Low ESS and hyperplipidemia were independent predictors of the development of TCFA or INT or MIN, whereas the differentiation of early lesions to TCFA vs. INT was associated with hyperplipidemia and hyperglycemia (Fig. Table). Conclusion: The magnitude of low baseline ESS determines the severity and heterogeneity of atherosclerotic lesions and, in combination with systemic risk factors, predicts the development of TCFA. These findings provide a perspective for the identification of early stages of a high risk plaque, thereby enabling highly selective pre-emptive coronary interventional studies to prevent adverse coronary events.

The Lysine Binding Site in Kringle IV Type 10 Is Required for Apolipoprotein(a)-mediated Changes in Vascular Endothelial Cell Phenotype

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Previous studies indicate that endothelial dysfunction plays a critical role in atherogenesis. We previously demonstrated that apolipoprotein(a) (apo(a); the distinguishing protein component of the atherothrombogenic factor lipoprotein(a)) elicits rearrangement of the actin cytoskeleton and intimal proliferation through endothelial cells (HUVECs), characterized by increased central stress fiber formation and increased cell permeability. These effects are mediated by increased myosin light chain (MLC) phosphorylation via a Rho/Rho kinase-dependent signaling pathway. Apo(a) contains kringle (KIV and KIV domains similar to those in plasminogen; apo(a) contains 10 types plasminogen KIV-like sequences, followed by sequences homologous to the plasminogen KIV and protease domains. Several of the apo(a) kringle sites contain lysine-binding sites (LBS) that have been proposed to contribute to the pathogenicity of apo(a). Here, we tested recombinant apo(a) (r-apo(a)) variants containing both amino-terminal and carboxy-terminal domains of the molecule, and found that the effect of apo(a) on increases in MLC phosphorylation and HUVEC permeability can be attributed to the kringle IV type 10 (KIVD) domain, within the carboxy-terminal half of the apo(a) molecule. Accordingly, 17KAsp(full length apo(a) species with a mutation in the strong LBS in KIVD) does not elicit these effects, nor does 17k Apo(a) in the presence of the lysine analog epsilon-aminoacrylic acid. In keeping with our previous observations, the effects of apo(a) on MLC phosphorylation and EC permeability were abrogated by Rho/RO kinase inhibitors as well as the MLC kinase inhibitor ML-7. We have shown that 17k-apo(a) treatment of HUVECs enhances the Rho kinase-mediated phosphorylation of MLC phosphatase at Thr 850, which inactivates the phosphatase activity and leads to an increase in phosphorylation of MLC by MLC kinase; this effect was not observed using the 17kAsp variant. Taken together, our findings indicate that the strong LBS in apo(a) mediates all of our observed effects of apo(a) on HUVEC phenotype. Studies are ongoing to further dissect the molecular basis of these findings.

CCRF7 Is Functionally Required for Atherosclerosis Regression and Is Activated in Vivo by LXR

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We have developed a mouse model of atherosclerosis regression by changing, after plaque formation, the plasma level of the plaque from hyperlipidemia to normolipidemia by transplantoic aortic segments from apoe–/– mice to wild-type recipients (mixed environment). As a control, mice with hyperplipidemia were also transplant recipients to continue the plaque progression environment. In this model, we reported emigration of plaque from cells to regional and systemic lymph nodes after 3 days in the regression environment. During regression, the foam cells had features of dendritic cells (DCs). Because DCs require the chemokine receptor CCR7 for migration, we measured its mRNA and protein, and found foam cell expression of this factor to be induced, but only during regression. Further experiments using blocking antibodies to CCR7 demonstrated a functional requirement for it in regression. We have recently reported that LXR mRNA increases in foam cells during regression and were interested in the precise mechanism behind this. Using a murine model of in vivo DCs, we found that CCR7 expression is increased 8–9 fold upon LXR activation by the agonist T0901317. Importantly, this increase is dependent on CCR7 gene transcription, because pretreatment with actinomycin D abolished the observed response. To extend the results in vivo, we treated western-diet fed apoE–/– mice with LXR agonist. This also induced CCR7 expression in foam cells. Foam cell content and lesion area decreased by 21% and 24%, respectively, consistent with a report that LXR agonist promoted regression of atherosclerosis in LDLR–/– mice. The dependence of the agonist’s effects on CCR7 was demonstrated by treatment of apoE–/– CCR7–/– with LXR agonist: lesion content was only decreased by 9%. In conclusion, CCR7 is required in a mouse model for plaque regression with LXR being a factor that can modulate its expression.

Fibroblast and Aortic Smooth Muscle Cell Activation Is Regulated by Niemann-Pick Type C2 Protein

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Fibroblast and smooth muscle cell (SMC) phenotypic change represents a major underlying cause in several pathologies, including maladaptive vessel wall remodeling induced by hypertension, atherosclerosis, and angioplasty. Niemann-Pick type C2 (NPC2) gene has been recently identified as the second gene in the autosomal recessive cholesterol storage disorder, the NPC disease. It encodes a secretory, 151 amino acid protein with unknown function. This report identifies NPC2 protein as a novel autocrine/paracrine factor that negatively regulates fibroblast and SMC activation, thus playing an important role in regulation of aortic tissue remodeling. Our conclusions are supported by the following observations. First, loss of NPC2 protein in primary human dermal fibroblasts resulted in their activation. This was reflected by a strong increase in the expression of the collagen and inflammatory cytokine genes. The latter correlated strongly with the increased activity of NF-κB (~2.5-fold). Second, silencing of NPC2 gene in aortic SMC through siRNA transfection resulted in stimulation of the cell migration toward PDGF-β–/–. As a control, mice with hyperplipidemia were also transplant recipients to continue the plaque progression environment. These findings provide a solid basis for the development of novel therapeutics to treat the coronary artery and cerebral vascular diseases.

The Receptor for Advanced Glycation Endproducts Induces Cellular Migration via Binding of Its Intracellular Domain to Diaphanous-1: Implications for Vascular Biology and Disease

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Ligand binding to the Receptor for Advanced Glycation Endproducts (RAGE) activates a cascade of intracellular signaling events that lead to vascular dysfunction. The short cytoplasmic domain of RAGE is essential to activate these pathways, and hence the understanding of how
this domain functions in the intracellular environment is significant. In this study, we identified using the yeast two-hybrid assay that the RAGE cytoplasmic tail directly binds Diaphanos-1 (Dia-1); a molecule that mediates intracellular signaling and cellular motility. Evidence supporting this interaction is as follows: First, co-immunoprecipitation (IP) and binding of epitope tagged RAGE tail and Dia-1 was confirmed in transfected cells. Second, RAGE/Dia-1 was co-immunoprecipitated from RAGE-expressing cells, but not from cells depleted of RAGE tail deficient cells. Third, by confocal microscopy in intact cells, RAGE and Dia-1 co-localized after RAGE-ligand stimulation, with significantly less co-localization observed with DN-RAGE and Dia-1 expressing cells. To demonstrate the interaction between RAGE and Dia-1 in vivo, the interac- tion was identified in apolipoprotein E knockout mice, and its co-localization in aortic lesions and monocyte-derived cells. To test the biological relevance of the RAGE / Dia-1 interaction, functional studies were performed using siRNA to knockdown Dia-1 expression or scramble siRNA as control. Compared to siRNA scramble control, Dia-1 siRNA blocked RAGE ligand stimulated cellular migration and invasion. In conclusion, we have identified a novel signal transduction mechanism linking the intracellular function of RAGE to Diaphanos-1. We propose that blockade of RAGE/Dia-1 interaction represent a novel target for therapeutic intervention in vascular disease.

Multiplexed Immunoassays for Simultaneous Quantification of Soluble Cardiovascular and Metabolic Biomarkers in Mouse Blood

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A mouse is a key model in cardiovascular research. Limited blood volume has increased the difficulty of measuring multiple biomarkers necessary for understanding the biological processes associated with cardiovascular risk factors (e.g., atherosclerosis, inflammation and atherothrombosis). Using the Luminex™ xMAP technology, we developed multiplexed immunoassay panels requiring small sample volume for simultaneous quantification of multiple circulating biomarkers (e.g. apolipoproteins, adhesion molecules, absolute protein, and proinflammatory cytokines, etc) in mouse samples. Based on analytic compatibility, four separate multiplexed immunoassay panels were developed (Panel 1: sICAM-1, sVCAM-1, sSelectin, MMP-9, PA-1; Panel 2: Adiponectin, ApoE receptor A1, ApoE antibody, and Fibrinogen; Panel 3: 22 cytokines and chemokines; and CRP single-plex assay). Samples were analyzed using a Lumix™ Reader. Each antibody pair targeting an individual analyte is highly specific with no or negligible cross-reactivity to other analytes within the panel. The assay robustness is approximately 1:100 (Panel 1), 1:200 (CRP), or 1:5,000 (Panel 2). Thus, the total serum/plasma volume required for measuring all analytes is less than 55 µl for duplicate measurement. The assays may be used for other sample types (e.g. cell culture supernatant, tissue/cell lysate). These multiplexed immunoassays are reproducible and economic tools for simultaneous quantification of multiple CVD and metabolic biomarkers in mouse samples.

The Apolipoprotein(a) Present in the Carotid Artery Plaques of Subjects with High Lipoprotein(a) Reacts Against an Antibody Specific for Oxidized Phosphatidylcholine, Which Is Endowed with a Proinflammatory Potential

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Intriguingly, due to a substantial outward remodeling in younger mice (total vessel area increased collagen in the same region. These data suggest that the lack of apoER2 expression in the aortic roots decreases lipid accumulation, reduces SMC activation, and increases collagen production to yield a more stable fibrous plaque composition compared to the unstable lipid-rich lesions observed in apoER2 expressing LDLR-KO mouse. Despite differences in aortic root lesion composition, we were unable to detect significant difference in nitrosylated tyrosine residues and VACM expression in the aortic roots of LDLR knockout mice with or without apoER2. Interestingly, absence of apoER2 in male LDLR-KO mice significantly increased plaque formation in abdominal aortas compared to male LDLR-KO mice with apoER2 expression. The exaggerated size in abdominal aortas of apoER2-LDLR-dKO male mice was correlated to the presence of increased nitrosylated tyrosines compared to those observed in LDLR-KO mice with apoER2. Thus, apoER2 expression in the abdominal aorta may function to normalize endothelial function and protect against hypercholesterolemia-induced uncoupling of endothelial nitric oxide synthase.

Aging Induces a Decreased Vascular Remodeling Response in LDLR-/- Mice: Identification of Quaking as a New Player in Vascular Homeostasis

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Objective: Aging is regarded as a major risk factor for atherosclerosis. It is still unclear if it directly and/or indirectly impacts the process of atherosclerosis, and by which genetic pathways this is regulated specifically. Therefore, we not only evaluated the effect of aging in collagen induced atherosclerosis on lesion size, composition, and vascular remodeling, but also tried to identify differentially regulated genetic pathways in the context of atherosclerosis upon vascular aging. We used ApoER2-LDLR-/- mice as our atherosclerotic model system. Results: Collagen (40,500 vs. 14,800 µm²; P<0.04) and media areas (52,000 vs. 33,600 µm²; P<0.02) were larger in young vs. aged mice. In line with this finding, aortic root plaque area was larger in young mice (7.38×10ª vs. 5.67×10ª µm²; P<0.03). Intriguingly, due to a substantial outward remodeling in young mice (total vessel area 179,400 vs. 113,500 µm²; P<0.04), carotid lumen areas were increased compared to aged mice (86,572 vs. 65,122 µm²; P<0.04). No differences were seen in plaque composition, suggesting that age per se is not a causal factor in plaque stability. Microarray analysis revealed a significant up-regulation of the vasculessenescence pathway in aged mice, with specific up-regulation of the Quaking (Qk). Quk protein was detected in carotid root plaques and mainly observed in endothelial cells and macrophages. Quk function was subsequently assessed in vitro; gene silencing with Qk siRNA caused an increased wound healing response after scratching an endothelial cell monolayer (P<0.02), indicative of an impact of Quk in cell migration and/or regulation of/for inflammation in the atherogenesis and outward remodeling accompanying age increase in our collared induced atherosclerosis model. Qk seems to be an important player in age-dependent vascular homeostasis.

Protein Kinase C-delta Mediates PFGD-BB–Induced Stabilization of MCP-1 mRNA in Smooth Muscle Cells

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We have previously shown that PGDF-BB enhances monocyte chemoattractant protein-1 (MCP-1) mRNA stability in SMC by down-regulating a ribonuclease activity. To identify downstream targets involved in this action, we used a high-throughput strategy to screen a panel of PLC-dependent MAP kinase (MAPK) signaling inhibitors, phosphatases, and RNA helicases with 5×10⁴µM of actinomycin D (Act D) and PGDF-BB (5ng/ml) for 3 hrs in the presence of inhibitors of PGDF-mediated signaling pathways, and MCP-1 mRNA levels were measured by RT-PCR. The PLC inhibitor, P2R (sphingosine), PGDF-BB (5ng/ml) failed to block the effect of PGDF, whereas U73122, a phospholipase (PLC) inhibitor completely blocked the effect. We next examined...
Atherosclerosis is a complex disease resulting from the interactions of genetic and environmental risk factors leading to heart failure and stroke. We hypothesize that risk factors interact to alter expression of genes and pathways in the aortic wall and hence render it susceptible to atherosclerosis. Using an atherosclerotic mouse model (LDL-/ApoE-/- designated as LDL-), we performed microarray gene expression analysis to investigate candidate genes and pathways which are perturbed by the following criteria: genes (control C57BL/6 vs. LDL mice), eNOS stress (lesion-prone vs. lesion-resistant regions in LDL mice), diet (chow vs. high fat fed LDL mice) and age (2-month-old vs. 8-month-old LDL mice). Male C57BL/6 and LDL mice (n=16/group) were fed on either a chow or a high fat diet, sacrificed at 2- and 8-months-old, and RNA was isolated from the aortic lesion-prone and -resistant segments. Using Affymetrix Murine 430 2.0 chips (n=3), we profiled differentially expressed genes with the false discovery rate (FDR) p-value cutoff of 0.05 (fold change cutoff of 2). Then, we confirmed the normalization using two normalization methods: the invariant probe sets (dChip) and the quantile normalization (known as RMA), the statistical analysis was performed using t-test and ANOVA and pathway analyses (Pathway Express by Wayne State) were performed. The results revealed significant dysregulation of calcium signaling pathway in the LDL mice. Further elucidation of the signaling pathways through which PDBG-BB enhances MCP-1 mRNA stability may provide new approaches for inhibiting vascular inflammation and atherosclerosis.

Gene Expression and Pathway Analyses of Atherosclerotic Aorta Reveals Significant Dysregulation of Calcium Signaling Pathway

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Atherosclerosis is a complex disease resulting from the interactions of genetic and environmental risk factors leading to heart failure and stroke. We hypothesize that risk factors interact to alter expression of genes and pathways in the aortic wall and hence render it susceptible to atherosclerosis. Using an atherosclerotic mouse model (LDL-/ApoE-/- designated as LDL), we performed microarray gene expression analysis to investigate candidate genes and pathways which are perturbed by the following criteria: genes (control C57BL/6 vs. LDL mice), eNOS stress (lesion-prone vs. lesion-resistant regions in LDL mice), diet (chow vs. high fat fed LDL mice) and age (2-month-old vs. 8-month-old LDL mice). Male C57BL/6 and LDL mice (n=16/group) were fed on either a chow or a high fat diet, sacrificed at 2- and 8-months-old, and RNA was isolated from the aortic lesion-prone and -resistant segments. Using Affymetrix Murine 430 2.0 chips (n=3), we profiled differentially expressed genes with the false discovery rate (FDR) p-value cutoff of 0.05 (fold change cutoff of 2). Then, we confirmed the normalization using two normalization methods: the invariant probe sets (dChip) and the quantile normalization (known as RMA), the statistical analysis was performed using t-test and ANOVA and pathway analyses (Pathway Express by Wayne State) were performed. The results revealed significant dysregulation of calcium signaling pathway in the LDL mice. Further elucidation of the signaling pathways through which PDBG-BB enhances MCP-1 mRNA stability may provide new approaches for inhibiting vascular inflammation and atherosclerosis.

Ultrasensitive Confoal Imaging of C-Reactive Protein Binding to Fcγ-Receptors

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Background: C-reactive protein (CRP), the prototype human acute phase protein, is widely regarded as a key player in cardiovascular disease. Fcγ-receptors have been controversially discussed since 1999 to be receptors for CRP. Applying of FACS analysis and anti-CRP antibodies or radiolabeled CRP to resolve the CRP binding led to false positive results. Aim of the studies: Definition of CRP-binding to Fcγ-receptors applying highly sensitive technique. Methods: Using ultrasensitive confocal imaging analysis and newly labeled CRP we were able to overcome that problems. The technique enables us to perform observations and incubations on single native cells and precise association and equilibrium analysis. Results: Recently we showed that CRP binds FcγR-IIA (CD32) on transfected COS-7 cells. The avidity we assessed was ~100-fold lower than the one estimated for the anti-CRP/cRCP complex. In a work that followed up we demonstrated that CRP binding to COS-7 cells (CD32) on transfected COS-7 cells. The FcγRIIA was similar to that estimated for FcγR-I. In presence of γ-chain it increased up to 30-fold. It is important to note that the dissociation of CRP from the cell surfaces could not be detected over the time course of several hours and is thus extremely slow. Conclusion: Considering the permissive structural of CRP, we hypothesize that multivalent binding and receptor clustering are crucially involved in the interaction of CRP with nucleated cells. Actual state: We have been performing ongoing studies on monocytic cell lines and COS-7 cells transfected with FcγRII (CD16) and γ-chain that can enrich the conception of CRP binding to receptors on cellular membranes. We believe that the broader definition of CRP/Fcγ-receptor interaction will help CRP receptors can be of significant clinical importance for clarification of CRP pathological role in atherosclerosis and for the elaborating of efficient corresponding therapy.

Coronary artery disease (CAD) shows a complex disease pattern with regard to the extent and location of disease. The location of developing coronary artery plaques is of considerable importance as it significantly influences the myocardium at risk. Progression of atherosclerosis is of therapeutic value as well as prophylactic options. Recently, we described in a large collection of families with myocardial infarction that genetic factors contribute substantially to the location of the disease. In comparison to a disease manifestation in the distal segments, atherosclerotic disease at the ostium and proximal segments show a particularly high heritable component. The aim of this study was to characterize a hospital-based patient cohort with regard to disease and risk factor characteristics in order to better identify patients with higher-risk disease. We analyzed 411 coronary angiograms (201 male, 210 female; mean age 62.2 yrs) with regard to the location and degree of stenosis. The prevalence of an ostial lesion was 6.7% while 40.9% of the patients had lesions in the proximal segments and 3.4% exhibited lesions both in the proximal segments and the ostium. There was no significant difference with regard to age, gender, and risk factors such as hypertension, diabetes or hypercholesterolemia between patients with and without ostium stenosis. Patients with a proximal disease pattern were significantly older (69.0 yrs vs. 61.4 yrs; P=0.05) while there was no significant difference with regard to other risk factors. In a logistic regression including age, gender and classical risk factors, only age was a significant predictor of ostial disease (p=0.008). Further analysis for the degree of ostial stenosis confirms the significant influence of age (p<0.05). In conclusion, in this cohort of patients only a moderate higher age characterizes patients with ostium or proximal disease and classical risk factors do not allow for the identification of this high-risk patient population. In order to identify patients with ostial and proximal disease, novel disease markers would have to be identified. Given the heritability of this disease manifestation, we are currently pursuing genetic markers in addition to other non-traditional risk factors.
Glycemia, Triglycerides, and Disease Severity Are Best Associated with Higher Platelet Activity in Patients with Coronary Artery Disease

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Background: Although higher platelet activity has been described in patients with acute coronary syndromes consistently by many authors, consistent findings are reported about the relation of platelet activity to disease severity in stable patients with chronic coronary artery disease. Nevertheless, most reports studied only very small groups of patients. The aim of our study was to assess the relation of platelet activity to disease severity in sufficient number of patients with chronic coronary artery disease. Methods: One hundred and sixty stable patients with chronic coronary artery disease were studied (25 with single-, 63 with double- and 72 with triple-vessel disease). 95% of them were on aspirin. We compared patients who suffered from acute coronary syndrome. Platelet activity was determined as membrane expression of antigens CD62P (P-selectin, as % of positive cells) and CD41 (part of GpIIb/IIIa integrin, as mean fluorescence intensity) by flow cytometry. Platelet aggregability was measured with optical ADP aggregometry. Data sets were compared by Kruskal-Wallis test, correlation by Spearman test. Data are shown as mean ± SD. Results: Membrane CD62P expression correlated with vessel severity (p < 0.001, Kruskal-Wallis test). Patients with triple-vessel disease had the highest CD62P expression (1.81 ± 0.19) followed by patients with double-vessel (1.64 ± 0.22) and single-vessel (0.69 ± 0.09) disease. Positive correlation was found between CD62P expression with triglycerides (r = 0.49, p < 0.05) and CD41 with fasting glucose (r = 0.48, p < 0.05). No differences in ADP aggregability were found between groups. Conclusion: Higher platelet activity is present in patients with more severe coronary artery disease. More aggressive anti-platelet treatment in these patients should be considered, especially when metabolic syndrome is simultaneously present.

Human Prostacyclin Receptor Polymorphisms and Atherosclerosis

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Selective inhibitors of cyclooxygenase 2 (COX-2) confer a small but absolute risk of myocardial infarction and stroke presumably through inhibition of COX-2-dependent prostacyclin (PGI2). PG12 acts as a general repressor on endogenous stimuli to platelet activation, vascular proliferation and remodeling, hypertension, atherosclerosis, and cardiac function risk. Development of genetic biomarkers will be useful to identify the patients uniquely susceptible at developing risk of cardiovascular complications by selective inhibition of COX-2. We aimed to investigate the association between 31 distinct single nucleotide polymorphisms (SNPs) in the human PTGDR receptor (PTGR1) and the coronary calcium score (CCS), a surrogate measure for systemic vessel disease, in 40 individuals with a previous objectively confirmed deep vein thrombosis (DVT) and 21 controls, i.e. individuals without DVT but with comparable cardiovascular risk factors (cigarette smoking, diabetes, hypertensive status, lipid levels, body mass and systemic inflammatory status index, i.e. CRP, fibrinogen). We identified 5 SNPs which were not statistically significant different between the 2 groups (using Chi square test). Three were synonymous (no alteration in the coding amino acid sequence), V558I (DVT, 40% versus controls, 13%), V196 (DVT, 2.5% versus controls, 0%), S528S (DVT, 60% versus controls, 57%), and 2 were non-synonymous (change in the coding amino acid sequence), P226T (DVT, 2.5% versus controls, 0%), and R212C (DVT, 7.5% versus controls, 5%). Interestingly, R212C polymorphism is associated with functional deficiencies. In DVT and controls, IMT values were not significantly different (1.12 ± 0.25 and 1.13 ± 0.28 mm, mean ± SD). However, we found that the 4 individuals with R212C polymorphism (3 in DVT and 1 in control group) were characterized by significant (P < 0.006) higher IMT values versus all population (1.67 ± 0.37 versus 1.80 ± 0.37 mm). None of the 4 individuals were carriers of factor V Leiden. In conclusion, our results suggest a possible contribution of IP in the progression of atherosclerosis in humans and pave the way to perform larger studies assessing a possible correlation between R212C polymorphism and vascular disease.

Serum Total Bilirubin Level Is Inversely Associated with the Likelihood of Peripheral Arterial Disease: National Health and Nutrition Examination Survey, 1999–2004

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Background: Endogenous protective mechanisms that lessen susceptibility to peripheral arterial disease (PAD) are unknown. Bilirubin, a potent antioxidant and cytoprotectant, is a known antioxidant and cytoprotectant property of bilirubin and suggest that bilirubin diminishes susceptibility to PAD.

P354 Association of A-290G Polymorphism of CYP3A4 Gene in Response to Atorvastatin Therapy in Coronary Artery Disease

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The P450 cytochromes are super family of hemeproteins involved in the metabolism of various drugs and the isoenzymes show wide variation influencing both drug responses. Cytochrome 3A4 (CYP3A4) is the primary enzyme in the metabolism of Atorvastatin (lipid lowering drugs) and activity varies 10-folds in different ethnic populations. In the present study, we have examined the association of the functional variant in CYP3A4 gene in response to Atorvastatin in coronary artery disease (CAD) patients in North Indian population. It was a case-control study consisting of 101 CAD patients & 102 controls. We studied the single nucleotide polymorphism in the promoter region (A-290G) of CYP3A4 gene by Polymerase chain reaction - restriction fragment length polymorphism. The genotype frequencies of CYP3A4 gene were, AA: 33.67%, AG: 39.60% and GG: 26.73% in patients group and controls the frequencies were, AA: 59.65%, AG: 28.43% & GG: 11.78%. The frequency of homozygous mutant genotype GG was significantly higher in patients as compared to controls (p < 0.001). A significant reduction was seen in the levels after treatment with statin (p ≤ Standard error error

P355 Chronic Intermittent Hypoxia Induces Atherosclerosis in C57BL/6J Mice

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Introduction: Obstructive sleep apnea (OSA), a condition leading to chronic intermittent hypoxia (CIH), is associated with hyperlipidemia, atherosclerosis and a high cardiovascular risk. A causal link between OSA and atherosclerosis has not been established. Hypothesis: CIH may induces atherosclerosis in C57BL/6J mice. Methods: Forty male C57BL/6J mice; 6 – 8 weeks of age, were fed either a high cholesterol diet (HCD) or a regular chow diet (RD) and subjected either to CIH or intermittent air (IA) for 12 weeks. During exposure to CIH, FIO2 was reduced from 20.9 to 4.9 ± 0.1 % over a 30 s period and then rapidly reoxygenated to room air levels in the subsequent 30 s period. The CIH and IA states were induced during the light phase alternating with 12 h of constant room air during the dark phase. After the exposure, animals were sacrificed. The heart and proximal aorta were embedded in OCT and cross-sections were examined by Oil Red 0 staining. The descending aorta was examined in en face preparation stained with Sudan IV. Results: Nine out of ten mice simultaneously exposed to CIH and HCD developed atherosclerotic lesions in the aortic origin and descending aorta. In contrast, atherosclerosis was not observed in mice exposed to IA and HCD or in mice exposed to CIH and RD. HCD resulted in significant increases in serum total and LDL cholesterol (LDL-C) levels and a decrease in HDL cholesterol. Comparing to mice exposed to IA and HCD, combined exposure to CIH and HCD resulted in marked progression of dyslipidemia with further increases in serum total cholesterol (206 ± 8 mg/dl vs. 172 ± 12 mg/dl, respectively, p < 0.05) and LDL-C (124 ± 4 mg/dl vs. 106 ± 6 mg/dl, p < 0.05), a 2-fold increase in serum lipid peroxidation (malondialdehyde level of 1.33 ± 0.11 µM vs. 0.61 ± 0.05 µM, p < 0.05), and up-regulation of an important hepatic enzyme of lipoprotein secretion, stearoyl CoA desaturase 1. Conclusions: OH causes atherosclerosis in the presence of diet-induced dyslipidemia.

P356 Deficiency of Herp, an ER Stress Protein, Suppresses Atherosclerosis in apoE-deficient Mice

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Introduction: Herp is ER stress protein originally found in vascular endothelial cells. It is not expressed in the liver or in any organs other than the heart. Deficiency of Herp, an ER stress protein, Suppresses Atherosclerosis in apoE-deficient Mice.
Leptin Induces C-reactive Protein Expression in Vascular Endothelial Cells

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There is increasing evidence of an association between leptin and increased cardiovascular risk. Several studies have shown an independent interaction between high leptin levels and atherosclerosis, myocardial infarction, stroke, and coronary artery intima-media thickness, suggesting that high leptin levels imply increased cardiovascular risk. The mechanisms underlying the association of leptin with poor cardiovascular outcomes are not well understood. C-reactive protein (CRP) elicits proatherogenic effects in the vascular endothelium and positively correlates with leptin levels in humans. There have been no studies investigating the role of leptin in regulating CRP expression in vascular endothelial cells.

We tested the hypothesis that leptin induces CRP expression in human coronary artery endothelial cells (HCAEC) and sought to determine the signaling pathways involved. We confirmed the presence of both long and short isoforms of the leptin receptor in HCAEC. Incubation of HCAEC with leptin (0–400 ng/ml) caused a dose-dependent increase of CRP. mRNA and protein. This leptin-induced increased CRP expression was attenuated in the presence of anti-leptin receptor antibodies and also by inhibition of extracellular signal-regulated kinases1/2 (ERK1/2) by PD98059 (20–40 μM). Time (0–60 min) and leptin concentration (0–200 ng/ml)-dependent of ERK1/2 phosphorylation were evident in response to leptin treatment. Leptin (100 ng/ml) also elicited reactive oxygen species (ROS) generation. Inhibition of ROS by catalase (400 μg/ml) prevented ERK1/2 phosphorylation. Thus, leptin induces CRP expression in HCAEC via activation of the leptin receptor, increased ROS production and phosphorylation of ERK1/2. The local expression of CRP in HCAEC likely plays an important role in the development and progression of atherosclerotic lesions.

In conclusion, these studies suggest a mechanism for the proatherogenic effects of leptin. Leptin may provide a target for the development of preventive and therapeutic strategies against inflammatory mechanisms predisposing to cardiovascular disease.

Inhaled NO Potentiates Noncychel-mediated NO Dilation in Patients With Overlapping Autoimmune Diseases

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Accumulating evidence suggest that endoplasmic reticulum (ER) stress and unfolded protein response (UPR) contribute to several disease process including atherosclerosis. Increased expression of UPR target genes activates transcription factor 3 (ATF3) and ATF4 has been reported in human atherosclerotic lesions. Stimulation of the UPR induced with various ER stress inducers that require NO for egression of oxidized LDL. suggesting that lipid derived aldehydes could be involved in mediating ER stress and UPR. In the present study, we examined the role of endogenous aldehydes generated during phospholipid oxidation (e.g., 1,2-dihydroxy-3-oxo-phospholipid (POVP)) and 4-hydroxy-trans-2-nonenal (HNE) and acrolein, an environmental aldehyde in the induction of ER stress in human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAEC). Our data suggest, that these aldehydes (50 μM) cause the activation of double stranded RNA-activated protein kinase-like ER kinase (ERK) by 1.5–3.0 fold in endothelial cells. Moreover, HNE (10 μM) and POVP (10–25 μM) also cause the activation of CHOP in endothelial cells. In addition, L-homocysteine transport in both cell types was mediated by sodium-dependent and independent carrier systems. Competition studies revealed that the neutral amino acid transport system transport in cultured human aortic endothelial and aortic smooth muscle cells. In this study, we characterized L-homocysteine transport in cultured human aortic endothelial and smooth muscle cells. L-homocysteine was transported into vascular cells in a time-dependent fashion, L-homocysteine transport activity was about 2-fold higher in aortic smooth muscle cells. In addition, L-homocysteine transport in both cell types was mediated by sodium-dependent and independent carrier systems. Competition studies revealed that the neutral amino acids cysteine, glycine, serine, tyrosine, alanine, leucine, and methionine, and inhibitors of the cysteine transport systems inhibited L-homocysteine uptake in both cell types, but the inhibition was greater in endothelial cells. Endothelial-N2 cell line demonstrated that L-homocysteine transport in endothelial cells had a Michaelis constant (Km) of 79 μM and a maximum transport velocity (Vmax) of 10 μmol/mg protein/min. In contrast, homocysteine transport in aortic smooth muscle cells had a lower affinity (Km=212μM) but a higher transport capacity (Vmax=870 μmol/mg protein/min) (gt 6-fold). These studies suggest a mechanism for the proatherogenic effects of L-homocysteine uptake in endothelial cells.

Increased meal-induced oxidative stress in HIV-infected patients

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Increased meal-induced oxidative stress in HIV-infected patients may be a risk factor for cardiovascular disease. Although postprandial lipemia has been characterized in non-HIV, there is no detectable increase in postprandial peroxides associated with postprandial LDL, either in HIV or in non-HIV subjects. OS levels in non-HIV patients with documented CAD were also observed a transient fat-induced reduction in the levels of circulating antioxidants (AA) against malondialdehyde-modified LDL, mean normalized levels at 2, 4 and 8 hrs PP were 0.71 ± 0.16 (p<0.001), 0.85 ± 0.2 and 0.91 ± 0.12, respectively. Our data suggest that HIV+ patients have increased oxidative stress as demonstrated by acute changes induced by a standardized test meal enriched in PUFA. These meal-induced changes in oxidative stress may contribute to the risk for CVD in these patients.

Regulation of Homocysteine Transport in Vascular Cells

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Increased levels of plasma homocysteine is an independent risk factor for cardiovascular disease and has cell-type distinct proatherosclerotic effects on vascular cells. In this study, we characterized L-homocysteine transport in cultured human aortic endothelial and smooth muscle cells. L-homocysteine was transported into vascular cells in a time-dependent fashion, L-homocysteine transport activity was about 2-fold higher in aortic smooth muscle cells. In addition, L-homocysteine transport in both cell types was mediated by sodium-dependent and independent carrier systems. Competition studies revealed that the neutral amino acids cysteine, glycine, serine, tyrosine, alanine, leucine, and methionine, and inhibitors of the cysteine transport systems inhibited L-homocysteine uptake in both cell types, but the inhibition was greater in endothelial cells. Endothelial-N2 cell line demonstrated that L-homocysteine transport in endothelial cells had a Michaelis constant (Km) of 79 μM and a maximum transport velocity (Vmax) of 870 μmol/mg protein/min. In contrast, homocysteine transport in smooth muscle cells had a lower affinity (Km=212μM) but a higher transport capacity (Vmax=1200 μmol/mg protein/min) (gt 6-fold). These studies suggest a mechanism for the proatherogenic effects of L-homocysteine uptake in endothelial cells. The specific lysosomal function of L-homocysteine transport in endothelial cells may control cell type specific growth inhibitory effects and therefore play a role in homocysteine atherogenic potential.

Activation of the Endothelial S1P1 Receptor Inhibits Erk1/2 Phosphorylation in Type 1 Nondiabetic Diabetic Mice Through Induction of MAP Kinase Phosphatase-3

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Endothelial activation is a key early event in vascular complications of Type 1 diabetes. The non-obese diabetic (NOD) mouse is a well-characterized model of Type 1 diabetes. We recently
adjoining NCP than in other groups. Focal CP adjoining NCP may indicate the most advanced coronary atherosclerosis and may be important in the treatment of coronary artery disease.

### P365 Association of On-treatment Lipid Levels and Carotid Plaque Progression Assessed by Magnetic Resonance Imaging

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**Background:** Lowering of low-density lipoprotein cholesterol (LDL-C) via statin therapy has been associated with regression in coronary atherosclerosis. We examined the association between on-treatment lipid levels and changes to carotid plaques as identified by magnetic resonance imaging (MRI) after 2 yrs of treatment with the rosvastatin (RSV) therapy. **Methods:** Fortyeight subjects (70% men; mean age 65 yrs) with LDL-C ≥100 and <250 mg/dL and 16%–79% carotid stenosis by ultrasound were administered daily RSV (low dose [5 mg] or high dose [40/60 mg]) for 2 yrs. Multi-sequence, high-resolution carotid MRI at 1.5T was performed at baseline and annually. **Results:** In 33 subjects with matched baseline and 2-yr scans, RSV reduced total cholesterol (TC) by 34% (p < 0.0001), LDL-C by 50% (p < 0.0001), triglycerides (TG) by 12% (p < 0.01) and Apo B by 39% (p < 0.001). The mean LA, WA and NIH for the population as a whole showed a statistically significant decrease (p < 0.05). There was no change significantly different between subjects with the highest and the lowest VLDL-C. On a per subject basis, a 10% decrease in TC was associated with a 2.5% decrease in LA. Subjects with no significant change in TC had a 2.7% increase in LA. **Conclusion:** Greater lipid response to RSV therapy was associated with a reduction in plaque size. Individuals with less response to lipid-lowering therapy continued to have plaque progression. These findings support the idea of very intensive lipid lowering to achieve reduction in atherosclerosis.

### P363 Acute Changes in Shear Stress Induce Expression of Inflammatory Proteins in a Mouse Coarctation Model

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Blood flow has been identified as an important factor in the pathogenesis of atherosclerosis. Under disturbed flow conditions, wall shear stress (WSS) is transduced by the endothelium into a biochemical signal producing localized inflammation and plaque progression. There is evidence that acute changes in WSS can influence expression of inflammatory proteins. We evaluated the coronary arteries of 348 subjects using ECG-gated multislice CT. We calculated hemodynamic variables such as the magnitude of WSS, flow, and its turbulence properties. The coronary arteries were divided into 4 groups (1) with CP adjoining NCP, but with NCP alone or NCP separate from CP, (3) with CP without NCP, and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and (4) neither NCP nor CP. **Results:** In 33 subjects with matched baseline and 2-yr scans, RSV reduced total cholesterol (TC) by 34% (p < 0.0001), LDL-C by 50% (p < 0.0001), triglycerides (TG) by 12% (p < 0.01) and Apo B by 39% (p < 0.001). The mean LA, WA and NIH for the population as a whole showed a statistically significant decrease (p < 0.05). There was no change significantly different between subjects with the highest and the lowest VLDL-C. On a per subject basis, a 10% decrease in TC was associated with a 2.5% decrease in LA. Subjects with no significant change in TC had a 2.7% increase in LA. **Conclusion:** Greater lipid response to RSV therapy was associated with a reduction in plaque size. Individuals with less response to lipid-lowering therapy continued to have plaque progression. These findings support the idea of very intensive lipid lowering to achieve reduction in atherosclerosis.

### P364 Significance of Focal Coronary Calcification Adjoining Noncalcified Plaques Evaluated by Multislice Computed Tomography

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**Purpose** To determine the significance of focal coronary calcified plaques (CP) adjoining noncalcified plaques (NCP) using multislice computed tomography (CT). **Materials and Methods** We evaluated the coronary arteries of 348 subjects using ECG-gated multislice CT. We classified subjects into the following four groups: (1) with focal CP adjoining NCP, but without focal CP adjoining NCP, and (2) with CP with NCP, or (4) neither NCP nor CP, Groups were compared as to age, sex, coronary risk factors (RFs) and protein expression; 2.48 e-100 [40/80 mg]) for 2 yrs. Multi-sequence, high-resolution carotid MRI at 1.5T was performed at baseline and annually. **Results:** In 33 subjects with matched baseline and 2-yr scans, RSV reduced total cholesterol (TC) by 34% (p < 0.0001), LDL-C by 50% (p < 0.0001), triglycerides (TG) by 12% (p < 0.01) and Apo B by 39% (p < 0.001). The mean LA, WA and NIH for the population as a whole showed a statistically significant decrease (p < 0.05). There was no change significantly different between subjects with the highest and the lowest VLDL-C. On a per subject basis, a 10% decrease in TC was associated with a 2.5% decrease in LA. Subjects with no significant change in TC had a 2.7% increase in LA. **Conclusion:** Greater lipid response to RSV therapy was associated with a reduction in plaque size. Individuals with less response to lipid-lowering therapy continued to have plaque progression. These findings support the idea of very intensive lipid lowering to achieve reduction in atherosclerosis.

### P366 Withdrawn

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Notch signaling regulates cell fate in a variety of cell types and a role has been demonstrated for this pathway in angiogenesis. Epidemiological studies have reported various associations between alcohol consumption and several seemingly distinct diseases, including atherosclerosis, rheumatoid arthritis and certain cancers, all of which, however, involve angiogenesis. We assessed the hypothesis that ethanol (EtOH) modulates endothelial cell (EC) angiogenic activity via a Notch/CBF-1/RBP-Jk–Dependent pathway. EtOH and Notch receptor and Notch target gene mRNA and protein levels determined by qRT-PCR and western blot analysis, respectively. Network formation on Matrigel was used as an index of angiogenesis. Exposure of EC to EtOH (25mM, 24 h) significantly increased EC network formation; network length ≥0.22 vs 0.19 for control vs EtOH, respectively (n = 4, p < 0.05), while concomitantly increasing Notch receptor 1,3 and 4 mRNA and protein expression; 2.48 ± 0.33, 1.73 ± 0.2 and 3.77 ± 1.0 fold increase, respectively, for mRNA levels. In addition, Notch target gene (int-1 – 3) mRNA was significantly increased by 1.34 ± 0.52, 2.24 ± 0.34 and 2.78 ± 0.87, respectively. Selective knockdown of Notch 1 and 4 receptors by siRNA resulted in a significant decrease in EtOH-induced network formation;
Important Role of Erythropoietin Receptor in Promoting Vascular Endothelial Growth Factor Expression and Angiogenesis in Peripheral Ischemia in Mice

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Background: Prognosis of patients with severe peripheral artery disease (PAD) still remains poor when there are no indications of revascularization therapies such as bypass surgery or percutaneous transluminal angioplasty (PTA). It has been demonstrated that erythropoietin (Epo) treatment showed significant improvement in hindlimb ischemia. In this study, we examined the regulatory and paracrine action of Epo in endothelial cell (EC) gene expression.

Methods and Results: We assessed EC gene expression using Epstein-Barr virus encoded RPMS-1. These actions of ethanol may be relevant to the effects of alcohol consumption and disease progression.

Oxidized Low-Density Lipoprotein Induces Endothelial Progenitor Cell Apoptosis by Inhibiting PI3 Kinase/Akt Pathway via Tyrosine Nitration

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Objective: The mechanisms by which risk factors such as hypercholesterolemia reduce the number of circulating endothelial progenitor cells (EPCs) in patients with critical limb ischemia have not been fully clarified. We investigated the transcriptional effects of phospholipid oxidation by OxPL on the endothelial progenitor cell (EPC) functions.

Methods and Results: We demonstrated that OxPL treatment induces DNA-binding activity in cell extracts, which is associated with upregulation of PI3K and Akt phosphorylation. Furthermore, treatment with oxLDL significantly increased tyrosine nitration on p85 subunit of PI3 kinase A (PI3KA). These changes may explain the enhanced apoptosis displayed by EPC harvested from spontaneously hypercholesterolemic apoE-deficient mice.

Introduction: Critical limb ischemia in humans results from gradual arterial occlusion caused by atherosclerosis. Current mouse models of hindlimb ischemia use acute arterial occlusion that does not accurately mimic the pathogenesis of human chronic critical limb ischemia. We therefore developed the first mouse model of gradual arterial occlusion. Methods: Gradual arterial occlusion was induced by placing aeroform constractors on the proximal and distal femoral arteries. Blood flow recovery, ischemia-related gene expression, and neovascularization assays were performed for each animal model. Results: Blood flow recovery, ischemia-related gene expression, and neovascularization were significantly lower after gradual occlusion compared to acute occlusion. Conclusion: This novel model of gradual arterial occlusion more closely resembles the human condition, providing more accurate mechanistic insights with the objective of creating novel molecular therapies.

Fractalkine Induces Angiogenesis by Stimulating Vascular Endothelial Growth Factor-A Production by Human Vascular Endothelial Cells and Improves Hind Limb Ischemia

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Background: Angiogenesis facilitates the process of inflammation or improves ischemic condition. The present study investigated the detailed mechanism by which fractalkine (Fkn), a CXC3 chemokine, induces angiogenesis in vivo. Methods and Results: From the Fkn induced vessel sprouting from excised rat aorta and angiogenesis on chiko's cornealiooth chord-like membrane (CM) through CXC3R1 activation. Immunoblotting analysis and EMSA showed that Fkn upregulated hypoxia-inducible factor 1 (HIF-1alpha) by a mechanism, which is also upregulated in vivo. Therefore, we investigated the transcriptional effects of Fkn on the HIF-1alpha pathway in vivo. The results showed that Fkn induced HIF-1alpha expression in the femoral artery branches.

Conclusions: OxPL treatment inhibited PI3K/Akt activity and decreased phosphorylation of Akt, leading to increased apoptosis. Therefore, OxPL may be a potential therapeutic target for the treatment of atherosclerosis.

Current mouse models of hindlimb ischemia use acute arterial occlusion, which does not accurately mimic the pathogenesis of human chronic critical limb ischemia. We therefore developed the first mouse model of gradual arterial occlusion. Methods: Gradual arterial occlusion was induced by placing aeroform constractors on the proximal and distal femoral arteries. Blood flow recovery, ischemia-related gene expression, and neovascularization assays were performed for each animal model. Results: Blood flow recovery, ischemia-related gene expression, and neovascularization were significantly lower after gradual occlusion compared to acute occlusion. Conclusion: This novel model of gradual arterial occlusion more closely resembles the human condition, providing more accurate mechanistic insights with the objective of creating novel molecular therapies.
over 3 months. As a proof of concept, we mapped the VW contours of 5 subjects (3 atorvastatin, 2 placebo) and created thickness difference maps by subtracting the baseline and 3 month scan thickness maps. These maps show the spatial distribution of treatment-specific changes in wall and plaque thickness. Changes were observed mainly in the common carotid artery, with one of the subjects also showing changes in the internal carotid artery. In conclusion, this analysis showed that thickness maps generated from VW contours show location-specific changes over a three month period for the atorvastatin group. Further analysis of the entire study population using these maps will provide further insight into the temporal and spatial dynamics of plaque progression and regression.

**Phosphate Pathway, Where It Metabolizes Glucose 6-phosphate to 6-phosphogluconolactone**

Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway, where it metabolizes glucose 6-phosphate to 6-phosphogluconolactone and generates NADPH. In endothelial cells, NADPH is an essential cofactor for dihydrolipoate reductase, which generates tetrahydrobiopterin. Because tetrahydrobiopterin is an essential cofactor for endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial function. Increases in blood flow activate eNOS. We hypothesized that G6PD plays an essential role in flow-mediated eNOS activation. Using a core and plate viscometer, we showed that flow activated G6PD within 2 minutes. Small interference RNA knockdown of G6PD significantly inhibited flow-mediated eNOS phosphorylation. In contrast, overexpression of G6PD increased flow-mediated eNOS phosphorylation, while a G6PD mutant lacking enzymatic activity did not alter eNOS phosphorylation. Our hypothesis was further supported by data that G6PD expression increased significantly in cells exposed to laminar flow for 24 hours. In the Pretsch mouse, which has much lower G6PD activity due to decreased G6PD protein expression, aortic eNOS phosphorylation was dramatically decreased compared to wild type mice. These findings demonstrate a novel role for G6PD in vascular homeostasis, by regulating eNOS function.

**Omega-3 Fish Oil Plus Ginkgo Biloba Impairs Alzheimer Nanoplaque Formation and Size in Vitro**

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The relationship between Alzheimer’s disease and coronary artery disease is striking: Alzheimer plaques and arteriosclerotic plaques are basically very similar in their chemical composition. The principal constituents of Alzheimer plaques are proteoglycans, lipoproteins (preferentially VLDL and IDL), beta-amyloid and calcium, thus nearly extending by the component A-beta as compared to arteriосlesion plaques. Utilizing a patented arteriосlesion model (EP 0 946 676), we simulated in a biosensor assay Alzheimer plaques (J. Collid Interfac Sci. 276, 503–506 (2004)) and measured in vitro their formation and size by ellipsometric techniques, a laser-based physicochemical procedure. For the study with omega-3 fish oil plus ginkgo biloba (Probrin®, SevenSeas, Hull, England), VLDL apoE4/E4 from a cardiovascular and stroke-endangered high-risk patient was used as lipoprotein. Furthermore, human A-beta42 (0.1 g/L) which inclines strongly to aggregation and fibrillogensis, as well as EPA (21.9 mg/L), DHA (15.4 mg/L) and ginkgo biloba (0.28 mg/L) were applied in a concentration as could be expected in the blood of probationers after the intake of one capsule. The VLDL apoE4/E4 plasma fraction (30 mg/dL) showed beginning Alzheimer nanoplaque formation already at a normal blood Ca2+ concentration. Fish oil plus ginkgo applied acutely in the experiment, markedly slowed down this process of quaternary agglomerative nanoplaque complexation at all Ca2+ concentrations used. In a normal blood Ca2+ concentration of 2.52 mmol/L, the omega-3 + ginkgo-induced reduction of nanoplaque formation and molecular size amounted to 10.1 ± 1.3% (p < 0.03) and 14.3 ± 2.9% (p < 0.02), respectively, as compared to the controls. From these results, we concluded that the combination of omega-3 fish oil plus ginkgo hampered the docking of VLDL apoE4/E4 to the proteoglycan receptor and of A-beta42 to VLDL apoE4/E4, and that altogether Alzheimer nanoplaque formation is diminished as compared to control experiments without these substances. These in vitro experiments provide a mechanistic explanation for a possible beneficial mode of action of fish oil and ginkgo in Alzheimer, vascular or age-related cognitive decline.

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**Cardiovascular Targets of Dietary Isoflavones: Transcriptional Activation of Endothelial Nitric Oxide Synthase and Antioxidant Genes in Human Fetal Endothelial Cells**

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Estrogen protects premenopausal women against coronary heart disease, however, hormone replacement therapy following menopause has been linked with an increased incidence of breast cancer and limited protection against cardiovascular disease. Dietary soy isoflavones, such as genistein, daidzein and its metabolite equol, may serve as alternative estrogen receptor modulators to reverse endothelial dysfunction by increased synthesis the vasodilator nitric oxide.

In our recent study in human endothelial cells, we reported that equal rapidly (2 min) activates Akt and extracellular signal-regulated kinase (ERK1/2), leading to phosphorylation of eNOS Ser1177 and association of the enzyme with the chaperone Hsp90. We have now employed lucipherin chemiluminescence to document that genistein, daidzein and equol (100 nM) acutely stimulate superoxide anion (O2-) production in human umbilical vein endothelial cells (HUVEC). Pretreatment with the eNOS inhibitor, L-NAME (100 μM), abrogated equol stimulated O2- production (p < 0.01). Increased O2- production was associated with nuclear translocation of the transcription factor NFκB (2–4 h) and a 2-fold upregulation of the antioxidant stress protein heme oxygenase-1 (HO-1, n = 3, p < 0.05). Our findings suggest that isoflavones, at low nanomolar concentrations, evoke rapid release of both NO and O2-, leading to enhanced NFκB antioxidant response element transcriptional activation of HO-1 expression. Dietary soy isoflavones may thus afford protection against endothelial dysfunction in vascular diseases associated with prolonged oxidative stress.

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**Salt Inactivates eNOS: A Mechanism of How Salt Contributes to Hypertension**

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The contribution of a high salt diet to hypertension has been debated for decades. One pertinent detail to this debate is that the molecular mechanisms of how salt induces high blood pressure are not fully understood. Besides the kidney-volume system, vascular endothelial cells constitute an important mechanism for regulating blood pressure. Endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial and generates NADPH. In endothelial cells, NADPH is an essential cofactor for dihydrofolate reductase, which generates tetrahydrobiopterin. Because tetrahydrobiopterin is an essential cofactor for endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial function. Increases in blood flow activate eNOS. We hypothesized that G6PD plays an essential role in flow-mediated eNOS activation. Using a core and plate viscometer, we showed that flow activated G6PD within 2 minutes. Small interference RNA knockdown of G6PD significantly inhibited flow-mediated eNOS phosphorylation. In contrast, overexpression of G6PD increased flow-mediated eNOS phosphorylation, while a G6PD mutant lacking enzymatic activity did not alter eNOS phosphorylation. Our hypothesis was further supported by data that G6PD expression increased significantly in cells exposed to laminar flow for 24 hours. In the Pretsch mouse, which has much lower G6PD activity due to decreased G6PD protein expression, aortic eNOS phosphorylation was dramatically decreased compared to wild type mice. These findings demonstrate a novel role for G6PD in vascular homeostasis, by regulating eNOS function.

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**Glucose 6-phosphate Dehydrogenase Regulates Laminar Flow-induced Endothelial Nitric Oxide Synthase Activation**

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Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway, where it metabolizes glucose 6-phosphate to 6-phosphogluconolactone and generates NADPH. In endothelial cells, NADPH is an essential cofactor for dihydrolipoate reductase, which generates tetrahydrobiopterin. Because tetrahydrobiopterin is an essential cofactor for endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial function. Increases in blood flow activate eNOS. We hypothesized that G6PD plays an essential role in flow-mediated eNOS activation. Using a core and plate viscometer, we showed that flow activated G6PD within 2 minutes. Small interference RNA knockdown of G6PD significantly inhibited flow-mediated eNOS phosphorylation. In contrast, overexpression of G6PD increased flow-mediated eNOS phosphorylation, while a G6PD mutant lacking enzymatic activity did not alter eNOS phosphorylation. Our hypothesis was further supported by data that G6PD expression increased significantly in cells exposed to laminar flow for 24 hours. In the Pretsch mouse, which has much lower G6PD activity due to decreased G6PD protein expression, aortic eNOS phosphorylation was dramatically decreased compared to wild type mice. These findings demonstrate a novel role for G6PD in vascular homeostasis, by regulating eNOS function.

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**Not All Endothelial Cells Are Equal Under Flow: Studies on Cell Adhesion Molecule Expression**

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Endothelial adhesion molecule expression can be modulated by mechanical stimuli which are recognized through shear stress responsive elements that interact functionally with a number of transcription factors. As endothelial cells (EC) in the human cardiovascular system may exhibit multiple phenotypes in response to the complex flow patterns present in various vascular geometries, we examined the differential effects of venous-like (VF) and coronary artery-like flow (CAF) in EC. cigar-like cells were seeded on fibronectin-coated silicone rubber tubes and subjected to VF and CAF in a perfusion bioreactor. Immediately after 40h flow
Impaired endothelial barrier function can result from the release of inflammatory mediators in the cardiovascular system. We previously showed that human lung microvascular endothelial cells (HLMVEC) treated with cytokines (20 ng/ml interleukin-1β + 200 U/ml interferon-γ for 16 h) express kinin B1 receptors (B1Rs) and iNOS. Measurement of NO output in real time with a porphyrinic electrode showed that 1 mM Arg alone generated prolonged (90 min) iNOS-dependent “high output” NO (maximum = 295 ± 22 nM NO at 40–60 min) whereas addition of a B1 agonist, 100 nM des-Arg10-kallidin (DAK), produced “super-high output” NO (maximum = 32 ± 5 nM NO at 80–90 min) and an almost 10-fold increase over that of Arg alone. To investigate the hypothesis that B1R activation of iNOS-dependent NO production affects endothelial permeability, HLMVEC barrier function was continuously assessed by electric cell-substrate impedance sensing. Cytokine treatment of HLMVEC as assessed by electric cell-substrate impedance sensing. Cytokine treatment of HLMVEC as above markedly decreased resistance to half the control value. Addition of 1 mM L-Arg to stimulate basal iNOS activity resulted in partial recovery of cell resistance (25% increase from the post-cytokine level) whereas addition of B1 agonist 100 nM DAKO to acutely activate iNOS resulted in a significantly greater recovery (60% increase). ACE inhibitor enalaprilat (100 nM) also activated B1Rs and gave a response similar to that of DAK. This effect fully developed by 40–60 min after agonist treatment, lasted for at least 2 h and was blocked by B1 receptor antagonist des-Arg1-9-Leu-kallikrein or by iNOS-specific inhibitor N-(1-iminoethyl)-L-lysine. The role of NO was explored using the NO donor DETA-NONOate. Doses of DETA-NONOate (1 - 50 μM) that generated NO in the same range as B1R agonist stimulation resulted in a ~65% increase in resistance whereas doses caused no change (100 μM) or a further decrease (0.5 mM – 5 mM) in resistance. Thus, B1R activation of iNOS restores endothelial barrier function and could represent a novel target for repair of endothelial damage in inflammation. This mechanism could also underlie some of the beneficial therapeutic effects of angiotensin converting enzyme (ACE) inhibitors as these compounds are also direct agonists of B1Rs.

A Critical Role for NF-κB during FFA Impairment of Nitric Oxide Production in Endothelial Cells

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There is substantial evidence that endothelial vasodilation mediated by nitric oxide (NO) production is impaired in animal models of diabetes and in humans with type 2 diabetes. Increased free fatty acid (FFA) levels are often found in type 2 diabetes and are associated with endothelial dysfunction. We have previously shown that excess FFA, glucose, or TNF-α inhibits endothelial NO production. In endothelial cells, FFA activates iNOS, a regulatory kinase in the NF-κB inflammatory activation pathway and iNOS is both necessary and sufficient to impair insulin-mediated NO production. Objective: We hypothesized that NF-κB activation is necessary for FFA mediated impairment of endothelial insulin signaling. Results: Endothelial cells were exposed to 100 μM palmitate/BSA for 3 h and exhibited impaired insulin signaling and NO production compared to the BSA treated control condition. FFA treatment resulted in activation of the p38MAPK and NF-κB-dependent IκBα degradation. Overexpression of IκBα was confirmed by Western blot analysis. Control endothelial cells were transduced with a retroviral GFP construct. FFA treatment of HMEC transduced with the phosphorylation-resistant IκBα construct did not impair insulin signaling or insulin-mediated NO production when compared to the control GFP transduced cells. These experiments suggest that FFA-mediated impairment of NO production is dependent on NF-κB activation.

Recovery of Endothelial Barrier Function by Kinin B1 Receptor-induced Activation of Inducible Nitric Oxide Synthase

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Adiponectin and Its Receptors Increased Cholesterol Efflux in HEK293T Cells

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A decrease in adiponectin secretion causes the early stages of atherosclerosis. High-density lipoprotein (HDL) takes up cholesterol through ATP binding cassette A1 (ABCA1) as reverse cholesterol transport (RCT). Recently, a new therapeutic strategy, reconstituted (r)HDL, has been shown to enhance RCT. Therefore, we hypothesized that adiponectin increases the uptake associated with ABCA1 and also enhances HDL-induced uptake in human kidney cells (HEK293T), which endogenously expresses ABCA1. We transfected adiponectin receptor 1 and 2 (AdipoR1 and AdipoR2) cDNA into HEK293T cells. The transfected cells were labeled with 1H3-cholesterol following cholesterol loading with or without adiponectin for 24 hours. The levels of cholesterol efflux were analyzed using a liquid scintillation counter. Our results may give rise to the notion of vascular bed-specific endothelial molecular responsiveness: intrinsic differences in the vascular beds that serve as the source for different EC may account for the different effects of flow observed.

In Vivo Expression of Human Group II Secretory Phospholipase A2 Is Associated with Increased Production of Biglycan and Macrophage Colony Stimulating Factor

Mary Y Chang, Univ of Washington, Seattle, WA; Birgitta Rosengren, Mia Umaerus, Emelie Dagnelid, Eva Hurt-Camejo, AstraZeneca, Molndal, Sweden; Alan Chait; Univ of Washington, Seattle, WA

Secretory phospholipase A2 (sPLA2) digestion of lipoproteins leads to the formation of lysophosphatidylcholine (lysoPC), a molecule that we previously have shown stimulates the synthesis of biglycan and the proteoglycan form of MCSF (PG-MCSF) by arterial smooth muscle cells. These matrix molecules have pro-atherogenic effects. To test whether increased lysoPC synthesis of biglycan and the proteoglycan form of MCSF (PG-MCSF) by arterial smooth muscle cells after LPS injection 48hrs prior to sacrifice, or (iii) western diet

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Inhibition of Chemokine Receptor CCR2 in Bone Marrow Cells by siRNA Lentiviral Treatment Leads to Reduced Aneurysm Growth

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Introduction: Abdominal aortic aneurysms (AAA) form a relatively common vascular disorder among older men and can be lethal if left untreated. Treatment consists mostly of surgery when the expansion of the aorta has reached a certain diameter (5.5 cm). Before the aneurysms become this large, treatment is scarcely available. It has been shown that chemokine receptor CCR2 facilitates aneurysm formation in a murine AAA model, indicating that CCR2 receptor recruitment plays a role in AAA formation. Methods: siRNA Lentiviral supernatant containing the CCR2 siRNA vector (siiCCR2) or control vector. Lentiviral-transduced cells were harvested from donor ApoE−/− mice and transduced with concentrated lentiviral supernatant containing the CCR2 siRNA vector (siiCCR2) or control vector. Lentiviral-transduced cells (1.0x10⁷) were injected into irradiated recipient mice (single dose of 9 Gy X-ray total body irradiation). After 6 weeks the mice were fed a Western-type diet. During the last 4 weeks of diet, angiopoietin (Angi), 1.44 mg/kg/day) was given to induce AAA. Results: We observed 30% (3/10) premature death at the onset of AngII perfusion in the control mice and 18% (2/11) in the siiCCR2 mice. In the surviving mice 29% (2/7) of the control mice had outward remodeling of the aorta larger than 150% of the normal aorta diameter which qualifies as an aneurysm. In the siiCCR2 mice. In the surviving mice 29% (2/7) of the control mice had outward remodeling of the aorta larger than 150% of the normal aorta diameter which qualifies as an aneurysm. In the siiCCR2 mice silencing resulted in decreased aneurysm growth, showing that silencing of CCR2 by siRNA lentiviral vectors forms a therapeutic approach to inhibit aneurysm formation.

Conclusion: CCR2 gene silencing in CR2 was used to decrease aneurysm formation. For delivery of the CCR2 siRNA to leukocytes, a bone marrow transplantation experiment was performed in ApoE−/− male mice. Bone marrow cells were harvested from donor ApoE−/− mice and transduced with concentrated lentiviral supernatant containing the CCR2 siRNA vector (siiCCR2) or control vector. Lentiviral-transduced cells (1.0x10⁷) were injected into irradiated recipient mice (single dose of 9 Gy X-ray total body irradiation). After 6 weeks the mice were fed a Western-type diet. During the last 4 weeks of diet, angiopoietin (Angi), 1.44 mg/kg/day) was given to induce AAA. Results: We observed 30% (3/10) premature death at the onset of AngII perfusion in the control mice and 18% (2/11) in the siiCCR2 mice. In the surviving mice 29% (2/7) of the control mice had outward remodeling of the aorta larger than 150% of the normal aorta diameter which qualifies as an aneurysm. In the siiCCR2 mice silencing resulted in decreased aneurysm growth, showing that silencing of CCR2 by siRNA lentiviral vectors forms a therapeutic approach to inhibit aneurysm formation.

Role of Pericytes and PDGF-B in the Antileakage and Vascular Remodeling Actions of Angiopoietin-1

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Angiopoietin-1 (Angi) is a clinically relevant mitogen for endothelial cells with important anti-leakage and remodeling actions: it transforms distal capillaries of the microvasculature into enlarged vessels that are leakage-resistant in the setting of acute inflammation. In addition, Angi stabilizes blood vessels by promoting recruitment of pericytes during development. Recently, the anti-leakage effect of Angi was suggested to be due to its ability to reduce gap formation at intercellular junctions. However, little is known about the possible role of pericytes in this process. Abdominal coverage of blood vessels by pericytes is requisite for microvascular stability and is regulated by platelet-derived growth factor B (PDGF-B), a chemotactic factor promoting recruitment of pericytes to the microvasculature. To investigate the role of pericytes in the anti-leakage effect of Angi, we tested the ability of Angi to reduce inflammatory-induced microvascular leakage in the mouse trachea in the presence of a PDGF-B inhibitor. We also studied the effect of Angi and the PDGF-B inhibitor on pericyte recruitment by manipulation of CCR2 may lead to novel therapeutic approaches to prevent aneurysm growth. Method: siRNA-mediated gene silencing of CCR2 was used to decrease aneurysm formation. For delivery of the CCR2 siRNA to leukocytes, a bone marrow transplantation experiment was performed in ApoE−/− male mice. Bone marrow cells were harvested from donor ApoE−/− mice and transduced with concentrated lentiviral supernatant containing the CCR2 siRNA vector (siiCCR2) or control vector. Lentiviral-transduced cells (1.0x10⁷) were injected into irradiated recipient mice (single dose of 9 Gy X-ray total body irradiation). After 6 weeks the mice were fed a Western-type diet. During the last 4 weeks of diet, angiopoietin (Angi), 1.44 mg/kg/day) was given to induce AAA. Results: We observed 30% (3/10) premature death at the onset of AngII perfusion in the control mice and 18% (2/11) in the siiCCR2 mice. In the surviving mice 29% (2/7) of the control mice had outward remodeling of the aorta larger than 150% of the normal aorta diameter which qualifies as an aneurysm. In the siiCCR2 mice silencing resulted in decreased aneurysm growth, showing that silencing of CCR2 by siRNA lentiviral vectors forms a therapeutic approach to inhibit aneurysm formation.

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Anticonnective Tissue Growth Factor Antibody Attenuates Vascular Fibrosis and Prevents Passive Arterial Siffness

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Connective tissue growth factor (CTGF) mediates production of extracellular matrix (ECM) and vascular remodeling that facilitates development of atherosclerosis, arteriosclerosis and hypertension. We hypothesized that inhibition of CTGF might reduce vascular fibrosis and the stiffness of vessel wall. Vascular fibrosis was induced by 1%−nitro−l−arginine methyl ester (L−NAME) and high−fat diet. Also we studied the effect of Angi and the PDGF−B inhibitor on pericyte coverage of tracheal blood vessels. Using high−resolution confocal microscopic analysis we found, rather surprisingly, a 29% decrease in pericyte coverage in the microvasculature after Angi treatment, despite a 40% reduction in leakage. PDGF−B inhibition did not affect vascular leakage, even though pericyte coverage was reduced by 31%. Interestingly, PDGF−B inhibition in combination with Angi treatment resulted in a substantial increase in leakage by 61%, and a 55% reduction in pericyte coverage. Thus, Angi and the PDGF−B inhibitor had additative effects on pericyte loss and the anti−leakage effect of Angi was completely reversed by inhibition of PDGF−B. Our results suggest that pericytes are involved in the anti−leakage actions of Angi and that a threshold level of pericyte coverage may be necessary for vascular stability. They also indicate that crosstalk between Angi and PDGF−B pathways exists.

Circulating Inactive Human Matrix Gla Protein as a Biomarker for Cardiovascular Disease

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Matrix Gla protein (MGP) is a vitamin K−dependent protein and a strong inhibitor of vascular calcification. In both animals and humans MGP deficiency results in extensive arterial calcification. MGP activity depends on vitamin K−dependent carboxylation, hence vitamin K−deficiency leads to undercarboxylated MGP (ucMGP) which is inactive. Recently it was shown that ucMGP is strongly upregulated in calcified arteries, while in contrast local GaMP expression was very low. Therefore, we developed an ELISA−based assay with which ucMGP can be detected in the circulation. Serum ucMGP levels were measured in controls (n = 54) and in 3 patient populations, those with aortic stenosis (n = 25), chronic kidney disease (CKD 5D) (n = 40) and calciphylaxis (n = 25), chronic kidney disease (CKD 5D) (n = 40), chronic kidney disease (CKD 5D) (n = 40), chronic kidney disease (CKD 5D) (n = 40). Neither age nor gender influenced the circulating ucMGP concentrations. As compared to age− and sex−matched controls, all patient groups had significantly decreased ucMGP levels. However, in calcification−prone patients with renal failure virtually all patients had ucMGP levels below the control range. The renal patients underwent MSCT (multi−slice computed tomography) scanning of the coronary arteries. After stratifying the renal patients for tertiles of coronary calcification, ucMGP levels showed a significant decrease with increasing amount of vascular calcification: lowest tertile: 218 (79), intermediate tertile: 281 (89), highest tertile: 336 (118).
187 (46), and highest AS tertile 168 (49) mmol/L (p < 0.04). We conclude that the uGMSP assay clearly identifies cardiovascular patients and/or those at high risk for developing vascular calcification and that this assay may become an important tool in the diagnosis of cardiovascular disease.
shown that, infusion of Angl (1.00 ng/kg/min) into male C57Bl/6 mice decreased LRP protein abundance in the abdominal, but not the thoracic aortic region. We also demonstrated that Angl decreased the abundance of LRP and its intracellular chaperone, RAP in ex vivo aortic tissue in the abdomen, but not in the thorax. SMCs cultured from the abdominal region retained the property of Angl-induced reductions of both LRP and RAP (p < 0.05), which was attenuated by the AT1 receptor antagonist, losartan. Angl also reduced cell association and degradation of the LRP ligand, 125I-labeled alpha-2 macroglubulin, in cultured abdominal SMCs, but not in thoracic SMCs. This reduction was attenuated by the AT1 receptor antagonist, losartan. Real-time PCR demonstrated that Angl reduced abundance of RAP, but not LRP mRNA. In addition, Angl also reduced RAP protein and mRNA abundance in abdominal SMCs deficient in LRP. To investigate if Angl-induced reductions in RAP causes premature proinflammatory degradation of LRP, experiments were conducted with cyclohexamine (protein synthesis inhibitor) and a combination of cyclohexamine and MG-132 (proteasomal inhibitor). Angl reduced LRP protein in the presence of cyclohexamine, but not in the presence of MG-132, whereas RAP protein expression was inhibited even in the presence of MG-132 (p < 0.05).

Conclusions: Angl decreased expression of LRP in abdominal aortic SMCs in both ex vivo tissues and cultured cells in an AT1 receptor dependent manner. Angl reduced RAP independently of LRP, and caused degradation of LRP but not RAP, in a proteasomal dependent pathway. These data are consistent with Angl inhibiting RAP transcription to indirectly decrease cell surface expression of LRP.


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Introduction: G-protein coupled receptors (GPCR) modulate vascular tone, at least, in part by matrix metalloproteinases (MMP)-dependent epidermal growth factor receptor (EGFR) transactivation. We previously have reported that vascular alpha-1 adrenoceptor mediated activation of MMPs, such as MMP-7, and the EGFR mediates mitogenic rod processes to maintain vascular tone. In the present study we investigated the hypothesis that maintenance of adrenergic vascular tone by the MMP-EGFR pathway involves mitochondrial ATP synthesis. Methods and Results: In rat vascular smooth muscle cells (VSMCs), stimulation of alpha-1 adrenoceptors with phenylephrine triggered ATP synthesis in a concentration and time dependent manner. The increase in ATP synthesis was blocked by inhibitors of mitochondrial ATP synthesis (oligomycin), MMPs (GM 6001) and phosphoinositide-3-kinase (PI3K)- dependent Akt phosphorylation (wortmannin and LY 294002). Further, inhibition of MMPs or silencing EGFR expression blunted the phosphorylation of Akt and reduced GLUT4 translocation, effects that were also observed with PKC inhibitors. In small rat mesenteric arteries, exogenous ATP promoted activation of MMP-7, which was dependent on P2X but not P2Y purinergic receptors. Downstream of alpha-1 adrenoceptors, the activation of MMP-7 was blocked by inhibitors of ATP synthesis (oligomycin) or its bioavailability (apparex). Moreover, blockade of PI3K or ATP synthase dose-dependently inhibited adrenergic vascular tone. Conclusion: Modulation of mitochondrial ATP synthesis by MMPs and promotion of MPP activity by ATP are two new regulatory events in the signaling pathway of alpha-1 adrenoceptors. We suggest that metabolic and growth pathways merge to modulate MPP activity and thereby impact vascular tone. Supported by an operating grant from the CHF to CPP, PRN was supported by a program grant from the HSC and the MSFH, Canada.

Novel Effects of Lovastatin on Extracellular Matrix Gene Expression in Vascular Smooth Muscle Cells

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HMG CoA reductase inhibitors (statins) have been shown to reduce adverse cardiovascular events. Moreover, the cardioprotective effects of these agents do not appear to be limited to their lipid lowering capabilities. Other putative beneficial effects include inhibition of smooth muscle cell proliferation and migration. Piaque stability is another critical component of coronary and peripheral vascular disease. We examined the effects of a common HMG CoA reductase inhibitor, lovastatin, on the expression of plaque stabilizing genes including collagen type I (COLI), collagen III (COL III), lysyl oxidase (LOX), thrombospondin-1 (TSP-1), and tropoelastin (TROPO), in human umbilical artery smooth muscle cells (UASMC). In brief, UASMCs were incubated with 1μM lovastatin for 48 hours. RNA analysis included reverse transcription and quantitative real-time-PCR performed. Collagen I and III mRNA levels were not reduced (96 and 95%). Relative levels of LOX protein appeared slightly reduced in lovastatin-treated cells immunocytochemically stained with a LOX polyclonal antibody. Similarly, TROPO mRNA reduction appears to be related to transcriptional control in the MAPK pathway. Taken together, these results suggest a possible role for HMG CoA reductase inhibitors in extracellular matrix remodeling and plaque stability.
TRPM7 and its Kinase-sensitive Substrate Annexin-1 Are Differentially Regulated by Stretch and Vasoactive Agents in Vascular Smooth Muscle Cells

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Introduction: Transient receptor potential melastatin cation channel 7 (TRPM7) is important in vascular smooth muscle cell (VSMC) function. Mechanisms regulating TRPM7 are unclear. Hypothesis: Mechanical stretch and vasoactive agents, important in regulating vascular tone and growth, modulate TRPM7 in VSMCs. Methods: Rat mesenteric VSMCs were studied. Expression of TRPM7 mRNA and protein was assessed by RT-PCR and immunoblotting. Cellular localization of TRPM7 was evaluated by immunofluorescence microscopy. Activation of annexin-1, a TRPM7 kinase-sensitive substrate, was assessed by cytosolic-to-membrane translocation. TRPM7 was downregulated by siRNA. Cells were stimulated with vasoactive agonists, Ang II (10⁻¹⁰⁻¹⁰⁻⁷ mol/L), a vasoconstrictor, or bradykinin (10⁻¹⁰⁻¹⁰⁻⁶ mol/L), a vasodilator. Results: Immunofluorescence confocal microscopy demonstrated TRPM7 distribution along the cell membrane and co-localization with fibulin-2, marker of lipid rafts, in VSMCs. In the basal state TRPM7 was phosphorylated on serine/threonine residues. Ang II only modestly influenced TRPM7 expression and reduced phosphorylation. Bradykinin significantly increased TRPM7 expression and phosphorylation (2-3 fold) and TRPM7-induced Mg²⁺ influx (3-fold, p<0.05). These effects were abrogated in TRPM7 siRNA-transfected cells. Acute cyclic stretch significantly decreased TRPM7 phosphorylation by 50% and attenuated annexin-1 activation. Our results demonstrate that in VSMCs, TRPM7 is distributed in a network at the cell membrane and intracellularly where it associates with cholesterol-rich domains. Whereas mechanical stretch and Ang II tended to reduce TRPM7 activation, bradykinin upregulated TRPM7 expression and activity.

Conclusions: Our data suggest that TRPM7 is differentially regulated by vasoactive agents and stretch. Association with cholesterol-rich domains might facilitate TRPM7- and Mg²⁺- dependent signaling in VSMCs. These novel findings further characterize vascular TRPM7 and highlight the putative functional importance of this Mg²⁺ transporter in the regulation of VSMC function.

Intramural Delivery of Rapamycin with Molecularly Targeted Nanoparticles Inhibits Stenosis Without Delaying Endothelial Healing After Angioplasty

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Drug eluting stents have demonstrated the anti-restenotic benefit of local anti proliferative drug delivery following angioplasty. However, delayed re-endothelialization creates a risk for late in-stent thrombosis. Furthermore, small vessels may not be amenable for stent placement. We have shown that vüj3-integrin targeted perfluorocarbon nanoparticles deliver rapamycin into the arterial wall and reduce stenosis after angioplasty. The objective of this study was to determine whether intramurally targeted rapamycin nanoparticles impair endothelial repair. Femoral arteries of NZW rabbits fed an atherogenic diet for 4 months were subjected to balloon stretch injury. vüj3-integrin targeted nanoparticles with 0.3-mol% rapamycin in the surface were locally infused into one artery per rat by use of saline vüj3-targeted nanoparticles without rapamycin as controls. Microscopic morphometric analysis following Carstner’s staining showed that endothelial recovery following rapamycin treatment was not prolonged when compared with control at all time points (Figure). Intramural plaque area observed on end was significantly smaller (P<0.05) in arteries exposed to rapamycin nanoparticles than that in controls (Figure). In conclusion, constrained intramural delivery of rapamycin using vüj3- nanoparticles immediately after balloon angioplasty diminishes acute plaque formation without impairing endothelial healing.

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CD34⁺ Cells Stimulate Vascularization, Inhibit Inflammation, and Increase Cell Viability in Implantable Bioartificial Devices

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For successful engraftment of allogenic cells using a bioartificial device, the device must immuno-isolate cells, be immuno-invisible, exhibit good bulk transport characteristics, and be rapidly vascularized. Current devices are not vascularized quickly enough to maintain adequate cell viability and bulk transport, and device encapsulation is problematic. To address these issues, we tested devices consisting of a pro-angiogenic scaffold surrounding a cell-encapsulating alginate core in mice. Our goal was to prevent device encapsulation while stimulating vascularization. We found that despite their pro-angiogenic nature, the scaffolds acted as a physical barrier, inhibiting the approach of vessels to the alginate core. CD34⁺ peripheral blood cells promote vascular growth in diabetic mice, so we also examined their abilities. A scaffold with conditioned media was proven to be a better growth promoter than the device and reduced encapsulation. To determine if these translations to improved cell viability in devices, pancreatic β-cells were labeled with quantum dots. Retention of quantum dots (a measure of β-cell viability) was also increased by inclusion of CD34⁺ cells in the device or injection of the cells near the device. Because CD34⁺ cells from some patients are dysfunctional, clinically, it may be beneficial to use allogenic CD34⁺ cells. Thus, we immunosuppressed mice prior to device implantation and CD34⁺ cell injection. Surprisingly, immunosuppression, inhibited vessel growth and increased encapsulation. Thus, CD34⁺ cells may help create an optimal milieu for device implantation. Work supported by the Juvenile Diabetes Research Foundation

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ASS Nonresponder Rate Using Multiple Aggregation Tests Has High Prevalence in CABG Patients

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Object : The predominant mechanism of early graft failure after coronary artery by-pass grafting is associated with benefit of antiproliferate treatment of drugs like Asacys-salicicyc acid (ASA). The mechanisms for ASA resistance are multifactorial and can be screened by laboratory tests. Methods : We determined platelet function using whole blood impedance aggregometry (IA ) induced by ADP, arachidon acid ( AA ) and collagen ( Coll ), plateled aggregation ( P A ),

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To prevent the loss of blood following a break in blood vessels, components in blood and vessel wall interact rapidly to form a thrombus to limit hemorrhage. This hemorrhagic response is rapid and regulated, since excessive and inappropriate clotting reduces the patency of blood flow. During the last century many of the components involved in the hemostasis have been identified including proteins and cellular components in blood, elements in the vessel wall and hydrodynamic factors. Direct numerical simulation (DNS) is being used as a new tool to study biological processes. The aim of this study is to develop a meaningful computational model of clot development taking into account both physiological, biochemical and mechanical factors, which usually occur at different length scales. This involves the development of a Multiscale Computational Toolkit for Modeling Thrombus Development (MMTD). The proposed Toolkit MMTD builds upon a combination of FronTier, a software framework using a front tracking approach for the simulation of multiphase flows (developed by Dr. Glimm at SUNY at Stony Brook), and a Cell Potts Model-based (CPM) modeling environment, CompuCell3D (developed by Dr. Alber). The predictions of the model are tested by high resolution confocal monitoring of thrombus development following laser injury of the mesenteric vasculature. The model permits tracking platelet accumulation, fibrin deposition, leukocyte incorporation and hydrodynamic parameters. The comparison between predictions of the computational model and results of the experimental system permit refinement of the Toolkit MMTD. Initial studies suggest the importance of hydrodynamic parameters in the heterogeneous domain structure of a developing thrombus. The development of a computational model incorporating physiologically important parameters of thrombus development will enable one to identify rate limiting, critically important, regulatory parameters for which small perturbations result in large effects on thrombus development. Such improved understanding will make significant contributions in the development of therapeutic strategies to treat thrombosis and hemorrhage.

Molecular Characteristics of Inactivated Human Tissue Plasminogen Activator Interaction with Fibrin Implicate the Nature and Sites of the Binding Interactions

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Introduction. Recombinant tissue plasminogen activator (tPA) is the predominant thrombolytic agent employed for clinical treatment of myocardial infarction and ischemic stroke. The high-affinity tPA-fibrin interaction.

POLYMORPHISMS OF FACTOR XIII AND GENDER-RELATED DIFFERENCES IN RISK OF CARDIAC EVENTS AMONG POST-INFARCTION PATIENTS

Grzegorz Pietrasik, Wojciech Zareba, Daniel Ryan, Arthur J Moss; Univ of Rochester Med Cntr, Rochester, NY

Background. There is limited data on the effect of the FXIII V34L polymorphism in patients with coronary artery disease. Methods. We evaluated the effect of the Leu34 genotype of FXIII on risk of recurrent cardiac events (cardiac death, nonfatal MI or unstable angina) in a cohort of 1012 post-infarction (MI) patients: 819 with the Leu34 genotype and 393 without the Leu34 genotype. There were 760 men and 252 women; gene frequencies were similar across the genders (p = 0.73). Results. In total population, the Leu34 genotype was not associated with increased risk of event (adjusted HR = 1.18, p = 0.20). The Leu34 genotype was associated with higher rate of events among men, but not among women (Figure 1). After adjustment for clinical covariates, the Leu34 genotype remained risk factor for recurrent events in men (HR = 1.40, p = 0.03) but not in women (HR = 0.81, p = 0.38). Conclusions. The V34L polymorphism of FXIII gene is an independent risk factor for recurrent events among post-MI men but not among women.

The Intrinsic and Extrinsic Pathways in Hemostasis and Thrombosis

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Introduction. Coagulation is essential to prevent life-threatening hemorrhage but can be detrimental in instances of vascular thrombosis. Recent studies have indicated coagulation in hemostasis and thrombosis may be initiated by separate pathways. Interestingly, mice deficient in factors XII and XI of the intrinsic pathway exhibit major defects in thrombus formation following FeCl3 injury. Conversely, mice deficient in factor VII and tissue factor, the initiating cofactors of the extrinsic pathway, die during embryogenesis or perinatally from massive hemorrhage. The focus of this study is to compare the effects of the intrinsic and extrinsic pathways of coagulation in several models of hemostasis and intravascular thrombosis. Materials and Methods. Three animal groups were analyzed in this study; wild type, factor XII-inhibited, and factor VII deficient mice in a C57Bl/6 background. The injury models utilized include a vascular puncture model of hemostasis and two intravascular injury models; ferric chloride (FeCl3) and photochemical. The hemostasis model involves the puncture of the left carotid artery by a 30 gauge needle. Total bleeding times were manually recorded and hemoglobin absorption determined from collected blood. FeCl3 injury was performed by adding 3μl of 10% FeCl3 solution to a 5x102.2mm strip of filter paper, which was applied to the left carotid artery for 3 minutes. Blood flow was measured for 30 minutes post-injury. Rose bengal is a photoreactive dye that was administered intravenously and activated with a mercury lamp to induce thrombosis in mesenteric vessels. Thrombus formation was analyzed by measuring optical density of sequential images. Conclusion Factor XII-inhibited mice exhibited no defects in hemostasis but failed to develop occlusive thrombi following FeCl3 or photochemical injury. In contrast, NIH deficient mice deficient exhibited impaired hemostasis but minimal defects in thrombosis. These results suggest that FXII inhibition may provide a target for treating thrombosis without hemorrhagic complications.
Hematopoietic Cell–Derived Tissue Factor Accelerates Thrombosis in C-Reactive Protein Transgenic Mice

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Elevated plasma levels of C-reactive protein (CRP) are associated with increased risk of myocardial infarction, and transgenic mice expressing human CRP demonstrate accelerated thrombosis after vascular injury. However, the mechanisms underlying the prothrombotic activity of CRP are poorly defined. We tested the hypothesis that CRP promotes thrombosis via effects on TF expression by circulating cells of bone marrow origin. We irradiated male CRP-transgenic (CRP-Tg) mice and transplanted them with bone marrow cells (BMC) from wild-type (WT) mice or mice completely deficient in mouse TF, but expressing a human TF transgene at a very low level (approximately 1% normal human TF levels, termed “low-TF” mice). Six weeks after transplant we subjected mice to photochemical carotid artery injury and measured the time required to form an occlusive thrombus. Mean time to occlusion was 13.2 ± 2.3 min in CRP-Tg mice transplanted with WT BMC (n = 10) vs. 18.1 ± 2.1 min in CRP-Tg mice transplanted with low-TF BMC (n = 7, p < 0.08; additional transplant experiments are in progress). Plasma TF activity was significantly higher in CRP-Tg mice transplanted with WT BMC (n = 3) vs. CR-P-Tg mice transplanted with low-TF BMC (n = 4; 2.74 ± 0.19 pm vs 1.36 ± 0.18 pm, respectively, p < 0.03). In addition, WT BMC from CRP-Tg mice accelerated platelet aggregation to AA and collagen (n = 196) with no differences in thrombosis times compared to WT mice transplanted with low-TF BMC. These results suggest that CRP increases the risk of thrombosis by up-regulating circulating TF activity. They also support the hypothesis that circulating TF is prothrombotic, and may help to explain the association between elevated plasma CRP and increased risk of ischemic cardiovascular events.

The EP3 Receptor for Prostaglandin E2 in Peripheral Arterial Disease

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Background. Our genetic studies in Iceland link the PTGER3 gene encoding the EP3 receptor for PGE2 to increased risk for PAD. EP3 acts oppositely to the IP receptor for PGD2 to regulate cAMP in platelets. Production of PGE2 in atherosclerotic plaque may enhance platelet responsiveness to co-aggregants present in the damaged vessel wall, thereby tilting the balance of pro- and anti-thrombotic signals such that genetic variants in EP3 increase the risk for disease particularly in the arteries of the lower limbs as in PAD. Methods. Platelets were collected from 10 young controls, 20 patients with PAD, and 20 age-matched elderly controls. pVASP was measured by EIA. Results. EP3-dependent phosphorylation of VASP is the major check-point regulator of platelet cytoskeletal reorganization. Ioprost increases pVASP, preventing platelet activation, while sulprostone (an EP3 agonist) with a co-aggregant (e.g., collagen) decreases pVASP. The action of sulprostone is blocked by DG-041, an EP3 antagonist. Baseline platelet samples from young controls have approximately 2x greater levels of pVASP than do elderly controls or PAD patients (p < 0.0032). Thus, there is a strong, age-dependent decrease in platelet pVASP. PAD patients have 15% lower pVASP than elderly controls. Stimulation with ioprost increases pVASP > 100 fold, with both elderly and young controls reaching the same phosphorylation levels. However, ioprost increases pVASP in PAD patients significantly less (p < 0.01). Conclusions. Production of PGE2 in atherosclerotic plaque may push platelets to a more activated state characterized by decreased pVASP, in part due to signalling through the EP2/EP3 pathway. Our therapeutic hypothesis is that an EP3 antagonist may return platelets to a less activated state by restoring the balance of pro- and anti-thrombotic signalling through EP2/EP3 and PGD2/P pathays, respectively. Therefore, DG-041, a potent and selective EP3 antagonist, was developed as a novel antiplatelet agent and is currently in Phase II clinical studies.

EP3 Receptors: A New Target for Inhibition of Platelet Function in Inflammatory Thrombotic Disease

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P-selectin expression, Ca2+ mobilization and VASP phosphorylation. Sulprostone potentiated platelet function even in the presence of aspirin (an inhibitor of TXA2 synthesis) and AR-C69391 (a P2Y12 antagonist) and DG-O41 prevented this. Conclusions. EP3 receptors on platelets potentiate platelet activation and platelet interaction with blood leukocytes. An EP3 antagonist may be therapeutic value in inflammatory thrombotic disease.

Platelet Thromboxane Assay Is a Good Predictor of Aspirin Response

Andrew O Maree, Massachusetts General Hosp, Boston, MA; Patrick Dicker, The Royal College of Surgeons in Ireland, Dublin, Ireland; Ronan J Curtin, Cleveland Clinic, Cleveland, OH; Hani Jneid, Massachusetts General Hosp, Boston, MA; Peter Crean, Saint James Hosp, Dublin, Ireland; Dermot Cox, The Royal College of Surgeons in Ireland, Dublin, Ireland; Igor F Palacios, Kenneth A Rosenfield, Massachusetts General Hosp, Boston, MA; Desmond J Fitzgerald; Univ College Dublin, Dublin, Ireland

Introduction: Persistent normal platelet function despite aspirin therapy, referred to as aspirin resistance, has been associated with a threefold increased risk of major cardiovascular events. Hypothesis: We hypothesised that newer point-of-care aspirin resistance assays, such as the platelet function analyzer (PFA) -100, may be a less sensitive and specific measure of cyclooxygenase pathway inhibition when compared with laboratory assays of aspirin response. Methods: Platelet function in 276 stable cardiovascular disease taking aspirin (75–320mg) daily were screened by (PFA) -100 (collagen/epinephrine cartridge). Twenty-five aspirin-resistant and 25 matched aspirin-sensitive patients were recalled for further analysis. Assays of serum thromboxane (TX) B2, platelet TXB2 generation in response to exogenous arachidonic acid (AA) (platelet TX, urinary TX, platelet aggregation to AA (1.6mg/ml), epinephrine (SMu), collagen (0.5ug/ml) and (TRAP) (SMu) were performed. Results: Fifteen percent of patients were determined aspirin resistant, failing to prolong the PFA-100 closure times (>193 seconds). The PFA-100 correlated with weak but not strong platelet agonists or serum TX generation (Table 1). The platelet TX assay was the most sensitive measure of effective response to both weak and strong platelet agonists. Conclusion: In a stable cardiovascular population, the PFA-100 point-of-care assay correlated with weak but not strong platelet agonists or serum thromboxane B2 generation. Platelet thromboxane generation correlated closely with most assays of platelet function and may represent a sensitive assay of platelet cyclooxygenase inhibition and thus aspirin response.

TABLE 1. ASPIRIN ASSAY CORRELATION; RELATIVE RISK (95% CI); P-VALUE* FROM FISHER’S EXACT TEST.

<table>
<thead>
<tr>
<th>Assay</th>
<th>RR (95% CI)</th>
<th>P-Value*</th>
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<tbody>
<tr>
<td>PFA-100 (&lt; 193 sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum TX (&gt;2.2ng/ml)</td>
<td>1.19 (0.34 to 4.17)</td>
<td>0.7027</td>
</tr>
<tr>
<td>Platelet TX (&gt;900ng/ml)</td>
<td>3.69 (1.02 to 13.34)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urinary TX (&gt;130)</td>
<td>3.00 (0.68 to 13.27)</td>
<td>0.2404</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>7.62 (1.76 to 32.89)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Collagen Aggregation</td>
<td>(SMu/mg)</td>
<td>4.5 (1.53 to 13.23)</td>
</tr>
<tr>
<td>TRAP Aggregation</td>
<td>(SMu/mg)</td>
<td>1.61 (3.7 to 6.94)</td>
</tr>
</tbody>
</table>

Higher Platelet Activity Is Present in Patients with Restenosis After Percutaneous Coronary Intervention but Not in Patients with an Occlusion of Coronary Artery Bypass Graft

Pavel P Usmancik, Frantisek Bednar, Leona Pavkova, Petr Stros, Karel Jirasek, Petr Widimsky; CardioCentr, 3rd Med Sch, Charles Univ in Prague, Prague, Czech Republic

Background: platelet activity plays an important role in acute coronary syndromes as well as in the progression of atherosclerosis. The aim of the study was to compare platelet activity in patients with two different types of ischemic complications after coronary revascularization: an occlusion of an bypass graft after coronary artery bypass grafting surgery (CABG) and with a restenosis after percutaneous coronary intervention (PCI). Methods: Forty-five patients with coronary artery disease were studied in a cross-sectional designed study. Fifteen of them were patients with the worst bypass graft patency from Prague-4 study (control protocol-driven coronary angiography was performed one year after CABG). No patient was dual antiplatelet treatment at time of blood sampling. Platelet activity was determined by membrane expression of platelet antigen CD62P (P-selectin, % of positive cells) by flow cytometry, aggregability by ADP-aggregometry. Data are expressed as mean ± SEM. Results: All patients had similar risk factors, the normal risk factors of acute coronary syndrome. No patient suffered from acute coronary syndrome. Membrane expression of CD62P antigen was significantly higher in patients with restenosis compared to patients with occluded or patent bypass grafts (1.96 ± 0.07 vs. 0.77 ± 0.03 vs. 0.57 ± 0.03, p < 0.001, Kruskal-Wallis test). CD62P expression was not different between patients with occluded vs. patent grafts. ADP-aggregometry was not different between groups (55.5 ± 1.1 vs. 56.1 ± 0.8...
**Mechanism of Paradoxical Platelet Activation Induced by Blockers of Platelet Integrin αgβ3 (GPIIb/IIIa)**

Nicole Baselier, Baker Heart Roth Institute, Melbourne, Australia; Christoph Loeffler, Univ Hosp, Freiburg, Germany; Pierre Massignin, Yaping Yuan, Monash Univ, Melbourne, Australia; Meike Schwarz, Univ Hosp, Freiburg, Germany; Christoph Hagemeyer, Steffen U Eisenhardt, Ingo Ahrens, Baker Heart Roth Institute, Melbourne, Australia; Christopher Bode, Univ Hosp, Freiburg, Germany; Shaun P Jackson, Monash Univ, Melbourne, Australia; Karthikeya Peter, Baker Heart Roth Institute, Melbourne, Australia.

**Introduction:** αgβ3 blockers provide benefits when applied intravenously (although with limitations) but failed as oral drugs. We developed a model describing the current concept of ligand-mimetic integrin blockade as potential reason for paradoxical platelet activation.

**Methods:** Platelet activation was determined by flow cytometry, immunofluorescence microscopy and Ca2+ measurements. Results: As an experimental model, we show activation of platelets in solution induced by ligand-mimetic αgβ3 blockers consisting of 3 components: 1. Pre-stimulation (ADP 1 μM). 2. Induction of ligand-bound conformation of αgβ3 blockers, (binding of αgβ3 blockers, RGD-peptides and anti-LIBS antibodies) and 3. αgβ3 blocker-induced calcium (via antibodies). Platelet adhesion on collagen represents an in vivo correlate of platelet pre-stimulation and activation as detected by P-selectin expression (mean fluorescence ± SEM: no addition 6.13 ± 0.49 vs. epibatidine 18.25 ± 1.02, p < 0.001) CD63 and CD40L expression as well as by measuring Ca2+ ([Ca2+]i ± SEM: no addition 18.46 ± 4.65 vs. epibatidine 54.30 ± 10.62, p < 0.05). This paradoxical platelet activation can be inhibited by ADP (P2Y12) receptor blockers (e.g. clopidogrel).

**Conclusion:** We describe a mechanism of αgβ3 blocker-induced paradoxical platelet activation, which may explain major limitations of αgβ3 blockers. These findings suggest cost-medication with ADP-receptor blockers and moving beyond the initial approach of ligand-mimetic blockade towards the development of allostatic or activation-specific integrin blockade.

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**Assays Evaluating Platelet Inhibition Provided by Clopidogrel Yield Significantly Different Results and Correlate Poorly Among Themselves**

Chantal Parand, Marie Lordkipanidze, Thuy Anh Nguyen, Donald A Palisaitis, Hopital Sacré-Coeur, Montreal, Canada; Jacques Turgeon, Universite de Montreal, Montreal, Canada; Eric Schampaert, Jean G Diodati; Hopital Sacré-Coeur, Montreal, Canada.

**Background:** The level of inhibition of platelet aggregation provided by clopidogrel is subject to important inter-individual variations; however, it has been reported in various populations, using different platelet function tests, thus making the results difficult to compare. **Hypothesis:** We assessed the hypothesis that increasing the cumulative clopidogrel dose administered before PCI would result in better inhibition of platelet aggregation. **Method:** One hundred and twenty patients were prospectively randomized in a double-blind, placebo-controlled fashion into one of 4 groups of clopidogrel dosing regimens (1 week prior to PCI 300 mg on the day prior to PCI, 600 mg on the day prior to PCI, 300 mg followed by 75 mg daily oral clopidogrel for 1 week before PCI; and 300 mg followed by 150 mg daily started one week before PCI). Platelet function was assessed at baseline, at the time of diagnostic coronary angiography, and 2 hours after stenting by optical aggregometry (LTA) induced by 20 μM of ADP. **Results:** All regimens significantly reduced platelet aggregation at the time of angiography, as well as 2 hours following stenting when compared to baseline (p < 0.0001; Figure). The 300 mg bolus followed by 150 mg daily showed the greatest inhibition of platelet aggregation, while a single 300 mg bolus resulted in the least inhibition, an absolute difference of 30% at the time of angiography (p = 0.007), which increased to 36% 2 hours post-stenting (p = 0.007). Conclusion: In choosing a clopidogrel regimen, it is important to effectively block the surge in platelet activity induced by the PCI. The 300-mg bolus and 150-mg daily regimen seemed most effective in achieving and maintaining such a level of platelet inhibition.

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**TABLE: PEARSON’S CORRELATION COEFFICIENTS**

<table>
<thead>
<tr>
<th>TESTS</th>
<th>LTA, ADP 20 μM</th>
<th>WBI, ADP 5 μM</th>
<th>WBI, ADP 20 μM</th>
<th>Platelet count drop, ADP 5 μM</th>
<th>Platelet count drop, ADP 20 μM</th>
<th>PFA-100®, Verify Now®</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA, WBI drop, ADP 20 μM</td>
<td>0.94*</td>
<td>0.37*</td>
<td>0.47*</td>
<td>0.51*</td>
<td>0.53*</td>
<td>0.43</td>
</tr>
<tr>
<td>LTA, WBI, ADP 5 μM</td>
<td>0.36*</td>
<td>0.48*</td>
<td>0.40</td>
<td>0.53*</td>
<td>0.30</td>
<td>0.37</td>
</tr>
<tr>
<td>WBI, ADP 5 μM</td>
<td>0.92*</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.29</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>WBI, ADP 20 μM</td>
<td>0.11</td>
<td>0.06</td>
<td>0.27</td>
<td>0.31</td>
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</tbody>
</table>

*Correlation coefficient, n = 120

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**Prolonged Prophylactic Administration of High-dose Clopidogrel Before Elective Percutaneous Coronary Intervention Is More Effective Than the Standard 300-mg Bolus in Inhibiting Platelet Aggregation**

Chantal Parand, Thuy Anh Nguyen, Marie Lordkipanidze, Donald A Palisaitis, Hopital Sacré-Coeur, Montreal, Canada; Jacques Turgeon, Universite de Montreal, Montreal, Canada; Eric Schampaert, Jean G Diodati; Hopital Sacré-Coeur, Montreal, Canada.

**Background:** Effective platelet inhibition at the time of PCI reduces the risk of periprocedural thrombosis. In patients with stable angina who undergo elective PCI, the issue of optimal loading dose and time of clopidogrel administration remains controversial. **Hypothesis:** We assessed the hypothesis that increasing the cumulative clopidogrel dose administered before PCI would result in better inhibition of platelet aggregation. **Method:** One hundred and twenty patients were prospectively randomized in a double-blind, placebo-controlled fashion into one of 4 groups of clopidogrel dosing regimens 1 week prior to PCI (300 mg on the day prior to PCI, 600 mg on the day prior to PCI, 300 mg followed by 75 mg daily oral clopidogrel for 1 week before PCI; and 300 mg followed by 150 mg daily started one week before PCI). Platelet function was assessed at baseline, at the time of diagnostic coronary angiography, and 2 hours after stenting by optical aggregometry (LTA) induced by 20 μM of ADP. **Results:** All regimens significantly reduced platelet aggregation at the time of angiography, as well as 2 hours following stenting when compared to baseline (p < 0.0001; Figure). The 300 mg bolus followed by 150 mg daily showed the greatest inhibition of platelet aggregation, while a single 300 mg bolus resulted in the least inhibition, an absolute difference of 30% at the time of angiography (p = 0.007), which increased to 36% 2 hours post-stenting (p = 0.007). Conclusion: In choosing a clopidogrel regimen, it is important to effectively block the surge in platelet activity induced by the PCI. The 300-mg bolus and 150-mg daily regimen seemed most effective in achieving and maintaining such a level of platelet inhibition.

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**Altered Reactive Oxygen Species Generation and Scavenging in Platelets from Patients with Heart Failure**

Ashish Shah, Eugenia Gkaliakogkou, King’s College London, London, United Kingdom; Julia DeCourcey, Ruth Buckley, King’s College Hosp, London, United Kingdom; James Ritter, Albert Ferro; King’s College Hosp, London, United Kingdom.

Heart failure (HF) is characterised by increased oxidative stress, which results in a reduction in bioactive nitric oxide (NO) as well as increased platelet activation leading to increased thrombotic events. Platelets have the capacity both to produce and to scavenge reactive oxygen species (ROS). However, the production and scavenging of ROS by platelets in HF has not been well characterised. We therefore sought to determine whether platelets from patients with HF have higher ROS production and/or defective ROS scavenging capacity. Twenty five HF patients (mean age 58.3 ± 3.3 years; 20 male, 5 female; 7/9/4 patients in NYHA classes I/II/III) were recruited from the 1/9/4 patients in NYHA classes I/II/III and 19 healthy controls of similar age and sex distribution (mean age 57.1 ± 2.9 years; 12 male, 7 female) were studied. ROS production was measured in gel-filtered platelets by pholasin chemiluminescence, in the presence of horseradish peroxidase, both at baseline and for 30 minutes after stimulation with collagen (8 μg/ml). Responses were expressed as arbitrary light units/1010 platelets. In other experiments, the decrease in light signal over 10 minutes was measured following the addition of gel-filtered platelets to Tyrode solution, as a measure of ROS-scavenging capacity, and this was expressed as percentage decrease in luminescence. All data were expressed as mean ± SEM, and were analyzed by paired or unpaired Student’s t test as appropriate, with p < 0.05 (two tailed) taken as significant. Platelets from HF subjects exhibited greater ROS production than did those from controls, both basally (13320 ± 1665 vs. 8062 ± 1293 light units/1010 platelets respectively, p = 0.023) and after stimulation with collagen (36020 ± 9863 vs. 11120 ± 2176 light units/1010 platelets respectively, p = 0.036). On the other hand, the reduction in pholasin luminescence by platelets was less for HF subjects than for controls (65.9 ± 4.8 vs. 88.2 ± 2.3 % reduction in light signal respectively).
p < 0.0005. Our results suggest that, in HF, platelets produce more ROS both at baseline and after collagen stimulation, and also exhibit an impaired capacity to scavenge ROS. These factors may contribute to increased platelet activation, which in turn may contribute to the increase in thrombotic events observed in this condition.

**Introduction:** More than 500,000 percutaneous interventions are performed in the United States annually. Cardiologists and surgeons aim to maintain balance between hemostasis and thrombosis to prevent adverse events. However, peripheral arterial access complications occur in 1.5% to 9% of patients. **Hypothesis:** We assessed the hypothesis that a novel amphiphilic lipid compound would: 1) achieve hemostasis more effectively than control when injected into a swine liver biopsy tract and 2) inhibit common percutaneous procedure pathogens. **Methods:** Glycerol mono-oleate (GMO) is a cubic phase-forming amphiphilic lipid, which when exposed to excessive aqueous fluid at physiologic temperature, undergoes a spontaneous phase transition from a fluid-like lamellar phase to a cubic phase. Seven anticoagulated swine (ACT > 250 s) underwent ten open liver biopsies with a 14-gauge needle: five injected with GMO, and five injected with nothing (control). Thirty seconds, 2 minutes, 5 minutes and 10 minutes after the procedure, bleeding was objectively graded; 0 = no bleeding and 1 = bleeding. Also, GMO was injected into four plates containing culture media for Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae. When injected, GMO converted to a cubic phase with definitive margins in the culture media. Each bacterium was then coated over their respective media and GMO. **Results:** There was a significant (p < 0.017) treatment effect on each success/failure bleeding outcome at 30 seconds (p < 0.0001), 5 minutes (p < 0.0001), and 5 minutes (p < 0.0038) based on a multiple logistic regression analysis controlling for initial bleeding, pig, and liver biopsy site. At 10 minutes, the bleeding results were not significant (p = 0.0817), likely explained by a pigs innate ability to clot at this time period. For the bacteria experiment, there was no growth of bacteria on the GMO for any of the bacteria. Specifically, the Staphylococcus aureus plate displayed a 200 micron halo confirming no bacterial growth surrounding the GMO. These results illustrate a significant biosealant effect at multiple time points using GMO. Furthermore, GMO acts as a bacterial deterrent especially with Staphylococcus aureus.

Lack of von Willebrand Factor Release with Different Blood Flow Patterns Generated During Rhythmic Lower Limb Exercise

Joaquin U Gonzales, Barry W Scheuermann; The Univ of Toledo, Toledo, OH

The potential for vascular endothelial cell injury caused by hemodynamic stress during physical activity in humans is unknown, but may provide insight into the flow stimulus that improves endothelial cell function following exercise training. The purpose of the present study was to assess the hypothesis that different muscle contraction-produced shear forces could stimulate the release of von Willebrand factor (vWF), a marker of endothelial cell injury. **Methods:** Eight healthy young men [25.6 ± 3.1 (SD) y] performed 20 min of single-leg knee extension exercise at two contraction rates: fast (FR, 11% duty cycle) and slow (SR, 50% duty cycle). To control for metabolic demand of blood flow, work rate was held constant (15.25 W) between FR and SR. Common femoral artery blood flow was measured at rest and during knee extension exercise using Doppler ultrasound. Rocket immuno-electrophoresis was used to measure plasma levels of vWF from venous blood collected by venipuncture before, immediately after, and 60 min following exercise. High-intensity cycling exercise was performed on a separate day as a control condition since previous studies have shown post-exercise increases in vWF with this mode of exercise. **Results:** The FR and SR exercise protocols produced significantly different blood flow patterns with the FR resulting in a larger retrograde flow component (>100 mW/m²) than the SR which consisted solely of antegrade flow. As a result, the magnitude of blood velocity oscillations experienced by the vascular endothelium was different between contraction rates (FR: 154.5 ± 54.1 vs. SR: 112.7 ± 21.6 cm/s, P < 0.05) with the average calculated shear rate being greater for FR than SR (383.1 ± 147.7 vs. 357.6 ± 127.3 s⁻¹, P < 0.001). Since workrate was held constant, mean blood flow was similar between FR (180 ± 30 s⁻¹) and SR (739.7 ± 70.1 s⁻¹, P = 0.05). Plasma levels of vWF were similar between FR and SR at rest and failed to change following exercise. In contrast, vWF increased by 16% (P < 0.05) in plasma collected at 60 min following cycling. **Conclusion:** The different levels of hemodynamic stress that accompany lower limb exercise, at least during moderate intensity, do not induce endothelial cell injury as assessed by plasma levels of vWF.

Physiological Testosterone Stimulates Tissue Factor Pathway Inhibitor Expression in Human Umbilical Vein Endothelial Cells Via the Androgen Receptor

Hong Jin, Yu-guang Li, Geng Peng, Dong-ming Wang; Shantou Univ Med college, Shantou, China

**Aims:** The aim of this study was to evaluate the effect of testosterone with varied concentrations on antigen and mRNA levels of tissue factor pathway inhibitor (TFPI) (released by human umbilical vein endothelial cell [HUVEC] and to investigate the mechanism of this regulation. **Methods:** HUVEC within 2–3 passages were cultured in 96-well plates and 25 mm flasks. The cells were incubated in the presence or absence of testosterone (3, 30, 3000, 30,000 nmol/L) for 48 h. After the incubation TFPI levels of media were measured by IMMUNOS Total Eisa kit. And RT-PCR was carried out to compare each group’s TFPI mRNA level. Then experiments were repeated with HUVEC incubated in androgen receptor antagonist (flutamide 10 μM/mL) for 3 h previously. **Results:** Testosterone at physiologic concentrations (3.3 μM/L) stimulated the secretion of TFPI significantly (P < 0.05). However, TFPI antigen and mRNA levels were markedly reduced at a larger dose (3000 nmol/L), Flutamide attenuated testosterone’s effects (p < 0.05). **Conclusion:** Our results demonstrated that testosterone, at physiological concentrations, has beneficial influence on hemostatic system by enhancing the antiagulant activity through stimulating the TFPI levels secreted by the endothelium, and that the vascular androgen receptor is involved in the processes. **Figure 1** Testosterone’s effect on TFPI gene expression in absence or presence of flutamide (n = 5, n = 4). (A) M, marker; 1, control; Lane 2, SRM T; Lane 3, 30M T; Lane 4, 3000M T; Lane 5, 30,000M T; Lane 6, F and SRM T; Lane 7, F and 3nM T; Lane 8, F and 30nM T; Lane 9, F and 3000nM T; Lane 10, F and 30,000nM T. (B): testosterone F: flutamide

ACE2 Confers Endothelial Protection and Attenuates Atherosclerosis

Fina Loren, Yi Pan, Adrian Quan, Gulin Wang, Hwee Teoh, Suboth Verma; St. Michael’s Hosp, Toronto, Canada

One of the main effectors of endothelial dysfunction is angiotensin-II (AngII), and pharmacological approaches to limit AngII bioactivity remain the cornerstone of current cardiovascular therapeutics. Recently, ACE2 has been identified as a critical negative modulator of AngII bioactivity, which serves to counterbalance the effects of ACE in determining net tissue AngII levels. Although ACE2 is highly expressed within the endothelium, the functional significance of this is unknown. Using gain and loss of function strategies respectively, we systematically evaluated the role of ACE2 in endothelial regulation and vascular inflammation. Furthermore, since atherosclerosis represents a clinically relevant end-point of progressive endothelial dysfunction we examined the potential of ACE2 to limit experimental atherosclerosis. Isolated aorta from ACE2-deficient mice exhibited impaired endothelium-dependent relaxation. Furthermore, over-expression of ACE2 in human endothelial cells promotes endothelial cell migration and tube formation, and limited monocyte and cellular adhesion molecule expression; effects that are reversed in ACE2 gene silenced cells. Endothelial cells isolated from ACE2 deficient mice exhibited impaired tube formation and monocyte adhesion. Importantly, ACE2 promoted capillary formation and neovessel maturation in vivo and profoundly reduced atherosclerosis in ApoEKO mice. Statins, an integral component of atherosclerosis treatment increased endothelial ACE2 expression. These data indicate a critical role of ACE2 as a mediator of endothelial homeostasis, a fundamental biological process, and suggest that ACE2-based treatment approaches may limit aberrant vascular responses and atherothrombosis.

Early Major Adverse Cardiac Events and Long-term Survival in Patients Treated with Bare Metal Stents and Drug-eluting Stents

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Background Randomized controlled trials have reported a similar procedural risk and a reduced rate of restenosis with DES when compared with BMS. We sought to examine the relative performance of BMS and DES in an unselected patient population referred for coronary revascularization. **Methods** We examined in-hospital and long-term follow-up data from unselected patients who underwent isolated primary revascularization by DES or BMS at our institution. Two case-matched groups were created using the BMS and DES patient’s propensity score. Early (30 day) MACE, including death, myocardial infarction, stroke and mid-term survival, using the Kaplan-Meier method, are reported. Outcomes were compared, using Chi-Square and Log-rank statistics. **Results** For 2101 patients receiving DES, a propensity matched comparator was obtained from the BMS population. Three-year follow-up data was obtained. Early MACE were more frequent after DES implantation (DES 3.7% vs BMS 2.65%; p < 0.01). This was attributable to more frequent peri-procedural myocardial infarction in the DES group compared with the BMS group (DES 1.54% vs BMS 0.43%; p<0.01). Despite the difference in early risk, Kaplan-Meier estimated mortality at 3 years was not different (DES 8% vs BMS 7.5%; p<0.01). **Conclusion** In this retrospective, propensity-matched patient population, peri-procedural myocardial infarction was more frequent after DES implantation when compared with BMS implantation. Long-term outcome was comparable in patients receiving either BMS or DES. Given the relative lack of difference in early risk reported from
Glutathione Peroxidase Activity in Cerebral Vasospasm After Subarachnoid Hemorrhage

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An oxidizing environment has long been implicated in the etiology of cerebral vasospasm (CV) after subarachnoid hemorrhage (SAH). This environment has been variously postulated to be generated by inflammation, hypoxia, or free radicals. Using human CSF we have previously shown that heme oxygenase 1 and lipid peroxide levels are increased with CV. Here, we directly coregulate CSF glutathione peroxidase (Gpx) activity with occurrence of CV after SAH in humans. Human CSF was obtained as part of standard of care in an IRB exempt manner from SAH patients with or without CV, and from one healthy hydrocephalus patient (control CSF). A commercially available Gpx activity kit (Zoetis/Meopharm Corp.) was used to assess activity, monoclonal human anti-Gpx was obtained from AbCam for the western blot analysis and other chemicals were obtained from Sigma. Gpx from vasospastic patients (CSFV, n=5), non-vasospastic patients (CSFNP, n=5) and non-hemorrhagic controls (control, n=10) were subjected to the following analyses: Gpx activity, Gpx protein levels, copper levels and iron levels. Hemoglobin, bilirubin and lipid peroxidation levels had previously been determined for the same patient samples. Gpx activity levels (uU/l) were 32 ± 2.9, 98 ± 9.1 and 341 ± 29.7 for Control, CSFV and CSFNP respectively. We then performed western blot analysis to determine whether the difference in activity can be attributed to different levels of Gpx protein. We found no significant difference in band intensity between the three groups. Hemoglobin concentrations did not differ significantly between hemorrhagic CSF groups. Both copper and iron can act as pseudoperoxidases (non-enzymatic peroxidation). Copper(I) concentrations (expressed as μg per g hemoglobin) did not differ significantly between CSFV and CSFNP (38.50 ± 5.6 vs. 40.78 ± 5.28). Similarly, Fe(III) concentrations (μM) were not significantly different in both SAH CSF groups (CSFV, 29.7 ± 9; CSFNP, 18 ± 29.6). These results suggest an increase in Gpx activity that is indicative of CV after SAH. Further studies will reveal if this observation can be predictive, or if Gpx could be a viable therapeutic agent for this serious complication.

Conclusion:

Glutathione Peroxidase Activity in Cerebral Vasospasm After Subarachnoid Hemorrhage

Heme Oxygenase-1: A Novel Key Player in the Development of Tolerance in Response to Organic Nitrates

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Objective-Nitrate tolerance is likely due to an increased production of reactive oxygen species (ROS) leading to an inhibition of the mitochondrial adenine dehydrogenase (ALDH), responsible for the nitric oxide metabolism, and to impaired nitric oxide bioactivity and signaling. We tested whether differences in heme oxygenase-1 (HO-1) induction might explain why PETN but not GTN therapy is devoid of nitrate and cross-tolerance. Methods and Results-Wistar rats were treated with PETN or GTN (10.5 or 6.6A/14-A/kg/min for 40). In contrast to PETN, GTN did not induce cross-tolerance or cross-tolerance as assessed by isometric tension recordings in isolated aortic rings. Vascular protein and mRNA expression of HO-1 and ferritin were increased in response to PETN but not GTN. In contrast to GTN therapy, NO signaling, ROS formation (as determined by chemiluminescence) and the activity of ALDH-2 (as assessed by an HPLC based method) were not significantly inhibited by PETN. Inhibition of HO-1 expression by apigenin induced tolerance to PETN whereas HO-1 gene induction by hemin prevented tolerance in GTN treated rats. Conclusions-HO-1 expression and activity appear to play a key role in the development of nitrate tolerance and might represent an intrinsic antioxidative mechanism of therapeutic interest.

Adequately Treated Type 2 Diabetes is Associated with Lower Wall Shear Rate of the Common Carotid Artery

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Introduction: Arterial sites with low wall shear stress (WSS) are more prone to the development of atherosclerotic plaques, as was observed in carotid arteries in subjects with atherosclerosis risk factors. Diabetes mellitus, a strong risk factor, could be modified by statins and angiotensin-converting enzyme inhibitors (ACEI). The aim of our study was to discover if type 2 diabetes mellitus (DM) subjects compensated by metformin, with established statin and ACEI therapy, still have lower WSS in common carotid arteries than healthy controls. Methods: We enrolled 26 compensated DM subjects aged 62 ± 10 years, treated by metformin, statins and ACEI for more than 6 months, and 16 age-matched healthy controls. Ultrasound examination was targeted to distal 1 cm of common carotid arteries, where maximal and mean velocities were measured. Internal diameter (ID) and intima-media thickness (IMT) were analysed by a professional using a semi-automatic method. WSS was calculated as a measure of WSS, calculated according to the following formula: WSR = 4x velocity/ID. Differences between groups were analyzed by unpaired t-test. Results: Diabetic subjects had significantly lower WSR, because of both thinner lumen and slower blood flow velocities. Lower WSR was accompanied by higher IMT. Conclusion Adequately treated subjects with compensated DM still have different atherogenic hemodynamic profile.

Combining Paclitaxel and Sirolimus Favorably Improves Endothelial Cytotoxicity and Platelet Proaggregatory Effect of Either Agent Alone

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Drug eluting stents (DES) have over the past few years emerged as a mainstay of percutaneous coronary intervention (PCI) due to its superior patency rate over bare metal stents. With widespread utilization of DES, late thrombotic risk associated with these stents has increasingly become a public concern. Paclitaxel and sirolimus are the two agents used in the currently available DES in the US. Our previous research has shown significant differences between the effects of paclitaxel and sirolimus on human coronary artery endothelial cells (HCAEC) and human coronary smooth muscle cells (HCASMC). We found that while paclitaxel exerted toxic effect on HCAECs, its systemic presence effectively blocked platelet aggregation. Conversely sirolimus was found to have cytotoxic protective effect on endothelium but caused slight platelet activation. We hypothesized that combining the two agents may lead to more desirable overall effects than either agent alone for DES. Methods In this study the platelet anti- and pro-aggregatory effects were tested using different dose combinations of both drugs. The cytotoxic effects were evaluated taking combinations of demographic and anthropometric variables as potential predictors.
Prediction equations thus developed were applied on validation data set (30%). RESULTS: The simplest equation for predicting %BF included age, gender, BMI, tripeps skinfold and waist circumference ($R^2 = 84.4\%$). Replacing BMI with weight and height reduced the overall variance ($R^2 = 86.4\%$). BMI showed a strong correlation with all abdominal fat subcomponents, but it best correlated with SCAT ($r = 0.79$). The most precise predictive equation for estimation of IAAT included age, gender, BMI, hip circumference and waist circumference ($R^2 = 52.1\%$). Waist circumference was the strongest predictor of IAAT ($r = 0.69$) and hip circumference of SCAT ($r = 0.80$). Irrespective of age and gender, BMI and hip circumference explained 66.8% variability in SCAT. CONCLUSION: The following predictive equations would be clinically relevant for different ethnic groups: 1) %BF = $0.22\times$ weight - $0.42\times$height. Clinically relevant for adult Asian Indians: 1) %BF = $0.22\times$ weight - $0.42\times$height - $0.29\times$waist circumference. 2) TAF = $-238.7 + 16.9\times$age + $934.2\times$gender + $576.7\times$BMI + $432.4\times$waist circumference - $441.1\times$hip circumference. 3) SCAT = $-5168.9 + 7.07\times$BMI + $519.7\times$hip circumference.

**P432** Diet-Induced Obesity in Male Apolipoprotein E-Deficient Mice Increases Adipose Serum Amyloid A Expression and Atherosclerosis
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The current epidemic of obesity affecting Westernized nations is accompanied by an increase in atherosclerotic diseases. The links between obesity and atherosclerosis include hyperlipidemia, diabetes and chronic inflammation, yet the mechanisms linking obesity to accelerated atherosclerosis are not fully understood, in part due to a paucity of models of obesity and accelerated atherosclerosis. Similar to humans, apolipoprotein E deficient (apoE/-) mice spontaneously develop atherosclerosis over their lifetime. Therefore, we sought to determine if diet induced obesity accelerated atherosclerosis in apoE/- mice. Eight week old male apoE/- mice were fed a diet containing 35% or 60% fat for 10 or 16 weeks. Comparing fed mice with fasting fed mice a high fat diet increased body weight gain (3.3 fold, $p<0.001$) and plasma total cholesterol levels (17%, $p<0.05$) compared to mice fed the low fat diet. Atherosclerosis was increased in high fat fed mice compared to low fat fed mice ($0.473 \pm 0.084$ vs. $0.307 \pm 0.062$ mm2, $p<0.05$). Additionally, adipose tissue factor levels increased in mice fed high fat diet compared with low fat diet. Adipose tissue factor is a candidate pro-inflammatory cytokine. Adipose tissue factor increases in obesity and may be a mediator of obesity induced atherosclerosis. These findings suggest a potential role for IFNg, a TH1 derived cytokine, in obesity induced atherosclerosis.

**P433** Role of Postconditioned Medium from Glucose Primed Endothelial Cells on SMCs
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**Objective:** Glucose is considered to be implicated in the development of cardio-vascular disease in diabetes. Changes interactions between endothelial and human arterial smooth muscle cells (HUVECs/SMCs) are not fully understood. Consequently, adhesion, proliferation, expression of integrins and collagen types from HUVECs/SMCs are studied after treatment with post-conditioned medium (post-CM) from HC glucosed primed HUEVCs (human umbilical vascular endothelial cells) in order to avoid direct effect of high glucose level and NO. Methods: Cell viability was obtained by MTT assay, obtained from fibroblasts, endothelial, and adipocyte adherent cells by post-CM treatment. Equimolar orbitals has no effect. Antibody to α₄β₁ or αᵥβ₅ integrins expression, elevated systemic SAA concentrations (2.6 fold, p<0.05). Moreover, SAA was associated with VLDL, LDL and HDL in mice fed the high fat diet compared to those fed the low fat diet, in which SAA was primarily associated with HDL. Thus diets enriched in saturated fat induce obesity accelerated atherosclerosis in male apoE/- mice, which may in part be mediated via increased adipose tissue SAA synthesis.

**P434** Are Fats Created Equal in Obese and Lean Mice?
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INTRO: Obesity is defined as the excessive accumulation of lipids in adipocytes. These lipid-filled cells were considered inert blocks until a discovery in 1995 revealed that they contained metabolism-regulating proteins which have profound effects on the development of diabetes. Fatty acids compose 65% of adipocytes (and triglycerides make up 98%), yet the major component of adipocites and its role in obesity is largely unknown. AIM/HYPOTHESIS: In this study, we assessed the hypothesis that a significant discrepancy exists between the fatty acid composition of obese and lean mice. METHOD: We obtained epididymal fat from 10 adult dd/db mice and 10 control wildtype mice. We extracted fatty acids with chloroform and added heptadecanoic acid as an internal standard. We then quantified fatty acid composition by gas chromatography equipped with an omega wax 250 capillary column. RESULTS: We found that levels of palmitic and stearic acid were significantly higher in the epididymal fat of obese mice, while the amounts of linoleic acid were lower compared to that of lean mice (refer to table below). CONCLUSION: In this study, we found that the adipose tissue of obese mice contains significant more saturated fatty acids and less unsaturated fatty acids than that of lean mice. PROSPECTIVE: The diverse types of fatty acids play important roles. Our study indicates a possible connection between fatty acid functions and obesity.

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<th>Palmitic Acid</th>
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<td>Obese</td>
<td>29.88 %</td>
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<td>Lean</td>
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* % is out of total fatty acid composition

**P436** Decreased Plasma Adiponectin Concentration in Major Depression
Leo Roberto, Di Lorenzo Giorgio, Tesauro Manfredi, Fortuna Enzo, Bianchi Francesco, Razzini Cinzia, Siracusano Alberto, Lauro Renato, Romeo Francesco; Univ Tor Vergata, Rome, Italy

**Objective:** Adiponectin is the most abundant adipose-derived plasma protein. Recently adiponectin levels have been linked to most variables of metabolic syndrome and conventional risk factors for cardiovascular disease. However, its relation with major depression is yet unclear. Therefore we examined plasma adiponectin levels in drug-naïve major depression subjects and healthy controls. Finally we confirmed that transient overexpression of SCOS3 in 3T3L1's attenuated insulin-resistant in 3T3-L1 adipocytes.

**Methods:** Plasma adiponectin levels were determined in 48 drug-naïve major depression patients (9.7±4.8 years, n=27) and 18 healthy controls. Adiponectin levels have been linked to most variables of metabolic syndrome and conventional risk factors, so we performed a univariate analysis. After finding a relation with depression, we compared adiponectin levels in healthy controls and major depression patients (9.7±4.8 years, n=11) and performed a multivariate analysis. Finally we confirmed that transient overexpression of SCOS3 in 3T3L1's attenuated insulin-resistant in 3T3-L1 adipocytes.

**Results:** Adiponectin levels (4.8±3.1 mg/L) were significantly lower in drug-naïve major depression patients compared with healthy controls (7.1±4.9 mg/L). Adiponectin levels in major depression patients (4.8±3.1 mg/L) were lower compared with healthy controls (7.1±4.9 mg/L). Adiponectin levels in major depression patients were lower compared with healthy controls. Adiponectin levels in healthy controls were lower compared with drug-naïve major depression patients.

**Conclusions:** These findings suggest a potential role for IFNg, a TH1 derived cytokine, in obesity and systemic insulin resistance.
Testosterone-dependent Endothelial Dysfunction Following Insulin Resistance Is Mediated by Regulation of 20-Hydroxyeicosatetraenoic Acid Synthesis

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Endothelial dysfunction plays a key role in the development of hypertension following insulin resistance (IR), a major cause for concern in today’s society. In high fructose-fed rats, a model of IR and hypertension, testosterone is essential for the development of endothelial dysfunction. Testosterone-dependent regulation of Cytochrome P450 (Cyp) 4A controls the synthesis of the arachidonic acid-derived vasconstrictor 20-HETE. Cyp2C-catalyzed epoxyscleratonic acids (EETs) and nitric oxide (NO) are vasodilators that counteract the actions of 20-HETE. As EETs and NO levels are decreased during IR, we suspected a role for testosterone-dependent vasoactive pathways in impairing endothelial relaxation. We hypothesized that following insulin resistance, testosterone-dependent alterations in 20-HETE synthesis contribute to the development of endothelial dysfunction and hypertension. To test this hypothesis, we conducted 2 separate studies on male Wistar rats. In study 1, sham-operated or gonadectomized rats were fed with 66% fructose for 9 weeks. Subsequently, the superior mesenteric arteries were isolated and assessed for changes in endothelium-dependent relaxation to acetylcholine in the presence or absence of 1-aminobenzotriazole (ABT), a Cyp4A blocker. In study 2, following 9 weeks of fructose feeding, rats were treated for 3 weeks with 25 mg/kg ABT (i.p.) or the anti-androgen flutamide (8 mg/kg s.c.). Blood pressure was measured prior to fructose feeding and at the end of 9 weeks. We also measured plasma testosterone and insulin resistance index. Fructose induced insulin resistance but did not affect testosterone levels. In vivo, ABT improved the relaxation to acetylcholine in the blood vessels of intact rats with IR. Both gonadectomy and flutamide decreased blood pressure in IR rats. Treatment with ABT did not affect insulin sensitivity but decreased the blood pressure. Further studies are in progress to identify and quantify the specific Cyp4A and Cyp2C isoforms associated with the IR-induced switch in vascular arachidonate metabolism. In conclusion, our studies point to the involvement of androgen receptor-mediated increase in 20-HETE synthesis in the development of endothelial dysfunction and subsequent hypertension.
Conclusions: Pathways of cholesterol efflux to apoA-I work synergistically and represent the predominant pathway of cholesterol export from macrophages in vitro and in vivo.

**P443**

**LCAT Deficiency Accelerates Cholesterol Accumulation of Liver in ABCA1 Null Mice**

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 Aim: To reveal the cholesterol homeostasis on hsp90a1/pchosteremia in mice, both 1) the diffusion mediated 'efflux' pathway which accelerate by LCAT reaction and 2) apoA1/ABCA1 mediated cellular cholesterol releasing pathway were abolished in mutant mice. Method: Six genotypes, wild, LCAT(-), ABCA1(-), ABCA1(-)/ABCA1(-), LCAT(-)/ABCA1(-)/ABCA1(-), of 22 week-old mice were examined. Euthanized mice were perfused with PBS with subsequent fixation by 4% formaldehyde. Lipid content of each tissue was measured by enzymatic color detection method (Kyowa Media). Results: Tissue TC of liver (43.3±8.7 mg/mg) from male mice were increased in LCAT(-) (134.7±10.4 mg/mg), ABCA1(-) (79.8±7.4 mg/mg) and in LCAT(-)/ABCA1(-) (197.3±18.3 mg/mg); Liver TC from female mice (44.7±6.6 mg/mg) were also modified in LCAT(-) (55.5±12.5 mg/mg), ABCA1(-) (62.5±24.6 mg/mg) and in LCAT(-)/ABCA1(-) (80.3±15.4 mg/mg). On the other hand, CE content in steroidogenic tissue was decreased in LCAT(-)/ABCA1(-). A good correlation between body weight and liver cholesterol content was also observed in all genotypes. No significant differences in 2C contents were observed between wild-type and homozygous deficient mice.

**P444**

**Triglyceride Alters Cholesterol Metabolism and ER-Stress Pathways in Cholesterol Ester–Laden Macrophage Foam Cells**

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Macrophage foam cells are prominent in atherosclerotic lesions. In late stage disease, much of the cholesterol accumulation in these foam cells is found in large, swollen, lysosomes. Tissue culture models using human macrophages incubated with various modified LDLs indicate that cholesterol accumulation within lysosomes can disrupt lysosome function leading to foam cells with significant lysosomal free and esterified cholesterol, similar to cells found in atherosclerotic lesions. The cholesterol is trapped and not accessible for efflux, even in the presence of strong efflux promoters. In the artery wall, however, the foam cells are bathed not only by modified LDLs but other lipid particles as well, including triglyceride-rich particles, such as VLDL. Little is known about how metabolism of these TRP might affect cholesterol metabolism and, specifically, the formation of cholesterol-rich macrophage foam cells. Our studies explore the effect of TRP on intracellular cholesterol metabolism. Results show that triglyceride (TG), delivered to the cell as a component of VLDL or TG-rich lipoprotein, reduces cholesterol ester (CE) accumulation by 50% in THP-1 macrophage foam cells. Reduced CE accumulation occurs in response to increased TG levels within the cell particularly within lysosomes. TG, delivered to the cell as a component of TRP, decreases the volume of lysosomes providing further evidence of increased lysosomal cholesterol clearance. Cholesterol accumulation in lysosomes inhibits acidification of lysosomes but lysosomal TG reduced this inhibition and maintained lysosomal acidity. The maintenance of an acidic lysosomal environment would enhance the degradation and clearance of internalized CE, facilitating the movement of cholesterol into the ER for export. The TG delivered to the cell can be utilized in the cholesterol efflux. However, the presence of excess TG within the macrophage also activates ER-stress proteins, such as CHOP and Grp78, and induces the phosphorylation of eIF2α, indicating the activation of the unfolded protein response. Our results show that excess TG in CE-laden foam cells have multiple effects, the balance of which would influence the atherogenic potential of the foam cell.

**P445**

**The Absence of abcg1 in Alveolar Macrophages Triggers Pulmonary Inflammation**

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The ATP-binding cassette transporter G1 (abcg1) effluxes cholesterol from macrophages and plays an important role in pulmonary lipid homeostasis. Deletion of abcg1 in mice results in pulmonary lipolysis, consisting of accumulation of lipid-filled type II pneumocytes containing altered surfactant composition, and changes in lipid metabolism genes. We hypothesize that alveolar and monocyte-macrophages contribute to pulmonary lipolysis in these mice by triggering inflammation in the lung. To study the early development of pulmonary lipolysis in abcg1-/- mice, alveolar and macrophage-macrophages and dendritic cells were isolated from the lungs of 10-week old mice by bronchoalveolar lavage (BAL). BAL was used for RNA expression analysis, cytokine bio-plex arrays or flow cytometry. Alveolar macrophages isolated from abcg1-/- mice were lipid-filled and larger in size. Furthermore, abcg1-/- alveolar macrophages showed 5-fold or greater increased expression of proinflammatory cytokines MIP-2, IL-6, and IL-1beta compared to wildtype mice. Flow cytometry analysis of abcg1-/- BAL lymphocytes showed a 10-fold increase (2,725 abcg1-/-/monocyte-macrophages, 265 wt) in the number of newly recruited monocyte-macrophages (C011c-low C011b-high MHCh low) compared to wild type prior to phenotypic onset of lipolysis. When abcg1-/- mice received an intraperitoneal injection of 2mg/kg of LPS, macrophages isolated in BAL after 4 hours showed increased expression of proinflammatory cytokines TNFalpha, IL-6, KC and increased expression of COX2, PGE2 secretion into BAL fluid was used using a Bio-plex suspension assay and KC and compared to wild type. These data suggest that alveolar macrophages contribute to the pulmonary disease of abcg1-/- mice by secreting more proinflammatory cytokines and recruited monocyte-macrophages to the inflamed lung in the very early stages of lipolysis. Thus, we provide a novel link between macrophage abcg1 function and regulation of inflammation. Understanding the role of abcg1 in alveolar macrophages and its link to inflammation will aid in developing therapies for various pulmonary diseases and other inflammatory diseases.

**P446**

**Proteomic Profiling of Prechylomicron Transport Vesicles Involved in Assembly and Secretion of ApoB48-containing Chylomicrons by the Intestine**

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Intestinal lipoprotein overproduction, caused by an overproduction of apolipoprotein B (apoB)-48-containing lipoproteins in the small intestine, is increasingly recognized as an important feature of dyslipidemia of insulin resistant states. Chylomicrons are transported from the endoplasmic reticulum (ER) to the Golgi using a specialized compartment called the prechylomicron transport vesicle (PCTV). In normal intestinal masters, the small intestine constitutively secretes small lipoprotein particles containing apoB-48 to prime for fat ingestion. However, in fructose-fed hamsters, lipoproteins are overproduced. Fructose-feeding induces the assembly of intestinal lipoproteins which may change the formation of PCTVs. In this study, we report proteomic profiles of PCTVs isolated from the enteric ER of normal, chow-fed hamsters, as well as the fructose-fed hamster; an established model of diet-induced insulin resistance. PCTVs were examined under both fasting and postprandial (fat load) conditions. Using tandem mass spectrometry and 2D gels, we have characterized the PCTV and developed proteomic profiles of PCVT-associated proteins from insulin-resistant and control enterocytes, with the intention of identifying proteins involved in insulin signaling attenuation and lipoprotein overproduction. A number of PCVT-associated proteins were found to be differentially expressed including factors involved in lipoprotein assembly, namely microsomal triglyceride transfer protein (MTP) and apoA-48, as well as proteins involved in vesicular transport, such as Sar1 GTPase and VAMP7. Enhanced expression of these markers was observed in the fructose-fed/insulin resistant state as well as following an oral fat load. Detailed proteomic profiles of intestinal PCTVs under various dietary conditions will be presented. We postulate that upregulated expression of Sar1 GTPase and MTP in the fructose-fed hamster intestinal enterocytes may contribute to increased PCTV assembly, and may have increased our understanding of the intracellular assembly and transport of nascent chylomicrons and potential cellular factors responsible for lipoprotein overproduction in insulin-resistant states.

**P447**

**PCSK9 and LDLR Trafficking in Hepatocytes**

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Genetic variation in the proprotein convertase PCSK9 contributes to inter-individual variation of plasma levels of LDL-cholesterol (LDL-C). Gain-of-function mutations in PCSK9 are associated with elevated levels of LDL-C, whereas loss-of-function mutations gene cause hypercholesterolemia. PCSK9 affects LDL-C level by altering the expression levels of LDLR receptors (LDLR) post-translationally. The mechanism by which expression of PCSK9 modifies LDLR number is not known. Lagace et al. (J Clin Invest 116:2995) have demonstrated that addition of PCSK9 to cells results in a decrease in LDLRds. To determine if PCSK9 acts at the cell surface, we examined the effects of PCSK9 on LDLR levels in primary hepatocytes from wild-type mice and from mice lacking ARH, an endocytic adapter protein required for internalization of LDLR. In primary hepatocytes from wild-type mice, treatment with recombinant PCSK9 markedly reduced cellular LDLR levels within 4 h. In contrast, no reduction in LDLR levels was observed in hepatocytes from mice that expressed no ARH, an adapter protein required for LDLR internalization. To determine the late fate of the LDLR after internalization, we performed immunofluorescence confocal microscopy at timed intervals after addition of PCSK9 to examine the distribution of LDLR in WI-FB cells. Prior to the addition of PCSK9, the LDLR was located predominantly on the cell surface. Within 1 h of addition of PCSK9 to the media, the immunodepleteable LDLr co-localized with an early endosome protein (early endosome antigen 1), followed by co-localization with a late endosomal (endolin 78) and lysosomal protein (Cathepsin D). Western blotting confirmed that cellular levels of LDLR were markedly reduced at 4 h in the PCSK9 treated cells. These studies are consistent with LDLR internalization being required for PCSK9 action and that PCSK9 promotes recycling of the LDLR from a recycling pathway to a degradative pathway.
A Polymorphism in the Protease-like Domain of Apolipoprotein(A) Is Associated with Severe Coronary Artery Disease

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Genetic variants reproducibly associated with severe coronary artery disease (CAD) could improve risk stratification and shed light on disease mechanism. In case-control studies of white subjects whose severity of CAD had been assessed by angiography, we previously tested 12,077 single nucleotide polymorphisms (SNPs) and found 5 SNPs that were nominally significant at the 0.05 level. The top 10 most enriched KEGG pathways included six immunity-related pathways including the natural killer cell mediated cytotoxicity (hsa04660, p-value: 3.68e-07) and leukocyte transendothelial migration (hsa04670, p-value: 8.06e-07) pathways, and four that were general biological pathways. The top 10 GO categories included six immune-related and four general cell signaling categories. Taken together, the gene expression patterns strongly indicated involvement of the immune system in AAA. A more detailed follow-up of changes in gene expression in the two most enriched KEGG pathways implicates these immune-related biological processes in the pathogenesis of AAA.

Variation at the PCSK9 and LDLR Locus, LDL Levels, and Heart Disease in PROSPER

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Genetic variation at the low density lipoprotein (LDL) receptor (LDLR) and the proprotein convertase subtilisin/kexin type 9 (PCSK9) genes loci has previously been reported to affect LDL cholesterol (C) levels and coronary heart disease (CHD) risk. Both PCSK9 and LDLR gene products are regulated by intracellular cholesterol levels and affect LDL clearance. In order to analyse the association between variation at these gene loci, LDL C levels, and CHD, we examined DNA from the PROSPER study, a prospective study of 5,044 men and women, who accrued a median 7.3 years of follow-up during which 438 cases of CHD had occurred. We used family-based association analysis, adjusting for age, sex, and smoking status. Our analysis found no statistically significant association with LDL-C or CHD. Further analysis suggests that a significant proportion of the variation in LDL-C is accounted for by environmental factors.
risk factors in a recently completed clinical trial of asthmatics. In patients with moderate to severe asthma, serum C-reactive protein levels (CRP) were significantly lower in the montelukast group (n=60) compared to placebo (n=73) after one month (1.7mg/L vs. 3.2mg/L, respectively; P < 0.008) and six months of treatment (2.3mg/L vs. 3.5mg/L, respectively; P < 0.04). All serum lipid levels were also significantly lower in both montelukast and theophylline groups compared to placebo (P < 0.05–0.001). However, these effects were primarily observed in individuals who were also using inhaled corticosteroids as monotherapy for asthma. We conclude that asthmatics taking montelukast and, to some extent, low-dose theophylline have lower inflammatory and lipid CVD risk factors. These observations suggest that inhibition of the 5-LO pathway may have potential use as a novel treatment strategy for CVD in asthmatics, and perhaps the general population, although the effects on high-density cholesterol levels should also be taken into consideration.

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Differential Regulation of C-Reactive Protein and Lipoprotein-Associated Phospholipid A2 in Human Inflammatory Syndromes

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Background. Levels of C-Reactive Protein (CRP) and Lipoprotein-associated phospholipase A2 (Lp-PLA2) are biomarkers of cardiovascular disease (CVD). However, studies comparing acute and chronic CVD provide conflicting results. We hypothesized that these biomarkers are differentially regulated during human inflammation. To test this hypothesis we examined CRP and Lp-PLA2 induction during two distinct inflammatory syndromes, experimental endotoxemia and acute coronary syndromes (ACS). Method: A human endotoxemia model (n=20, 50% male, 80% Caucasian, mean age 57.3±8.4, inpatient GCRC protocol) was used. Whole blood Lp-PLA2 mRNA levels (n=16) and plasma levels of CRP and Lp-PLA2 mass and activity were measured for 24 hours prior to and following intravenous administration of 3 ng/kg endotoxin (LPS). Time matched analysis of variance (ANOVA) was applied to this data. We also compared plasma levels of CRP and Lp-PLA2 in patients with ACS (n=223) to age, gender and race matched controls (N=249) without coronary artery disease, nested in a large angiographic cohort (N=3,800). Findings: Following LPS, Lp-PLA2 mRNA decreased transiently (ANOVA F = 6.77, p < 0.01) with greatest decrease of 82% at 2 hours (p < 0.001). Plasma levels of Lp-PLA2 mass declined by 18% at 6 hours, p = 0.007 although activity did not change significantly. In contrast CRP level increased by almost 10-fold (baseline 0.47 mg/L, peak 42.4 mg/L at 24 hours; F = 0.001) in endotoxemia. In either ACS or controls there was no correlation between plasma CRP level and Lp-PLA2 mass (r = 0.06, p = 0.73) in endotoxemia. In either ACS cases or controls there was no correlation between plasma CRP level and Lp-PLA2 mass or activity. In models adjusted for age, gender, race, Framingham Risk Scores, and medications plasma CRP levels (mean ± SD = 5.0 ± 4.3 mg/L) in ACS cases were compared to controls (4.71 ± 2.5 mg/L as 2.8 ± 2.5 mg/L; p = 0.001) whereas no significant difference was found in plasma levels of Lp-PLA2 mass (p = 0.10) or activity (p = 0.07) between ACS cases and controls. Conclusions: Unlike CRP, plasma Lp-PLA2 mass and activity as well as mRNA do not increase and may even decline during acute human inflammation. This differential in vivo regulation may account for conflicting epidemiological findings and influence utility as CVD risk predictors in different clinical settings.

The Role of Src Kinase in Regulating 15-Lipoxygenase Expression in Primary Human Monocytes

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IL-13 is a cytokine secreted by Th2 lymphocytes that is capable of inducing expression of 15-lipoxygenase (15-LO) in primary human monocytes. Our studies have defined the functional IL-13 receptor complex, interaction with JAKs and the receptor component and the tyrosine phosphorylation of specific Stat molecules, Stat1, Stat3, Stat5 and Stat6 in response to IL-13.

Stat1 and Stat3 serine 727 phosphorylation, an important step in IL-13-induced 15-LO expression. We demonstrated that Src is present in the signalosome along with p38MAPK and Stat1 and Stat3 serine 727 phosphorylation of specific Stat molecules, Stat1, Stat3, Stat5 and Stat6 in response to IL-13.

15-LO and p38MAPK are early regulators of hCRP gene expression. Studies to determine specific mechanisms and the impact of this pathway on hCRP transcription and inflammation.

Increased Interferon- Gamma Production in Response to Beta-2GPI Immunization in B Cell-Deficient Apeo-/- Mice

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B cells have been shown to be protective in atherosclerosis. Our laboratory and others have demonstrated that absence of B cells increases atherosclerosis in LDL-/- and apoE-/- mice. Immunization of atherosclerosis-susceptible animals with oxidized LDL elicits antibodies against modified lipoprotein and protects against atherosclerosis. Conversely, immunization of mice with another atherosclerosis-associated antigen, β2-glycoprotein I (β2-GPI) exacerbates atherosclerosis in a T helper cell-dependent manner. Because B cells have been shown to regulate T helper cell responses to specific antigen, we hypothesized that immune responses to β2-GPI by T helper cells could be regulated by B cells. To test this hypothesis, we compared the T helper cell immune response to β2-GPI in apeo-/- mice to apoe-/- mice deficient for B cells (μMT.apoe-/-). In these experiments, we immunized 10 apoE-/- and 10 μMT.apoe-/- mice with 10 μg of purified human β2-GPI with adjuvant (TiterMax Gold). Confluent mice received adjuvant only. Two weeks following the initial immunization, mice were boosted using the same protocol. Six weeks following the antigen boost, flow cytometry demonstrated no difference in absolute numbers of CD4+ T helper cells among the groups. However, compared to β2-GPI immunized μMT.apoe-/- mice, CD4+ T helper cells were significantly lower in atherosclerosis-prone mice (CD4+ 20% ± 3 vs 6% ± 1) and CD8+ (20% ± 3 vs 9% ± 2) T cells expressing the activation marker CD69. In vitro stimulation of splenocytes with 10 μg/ml of β2-GPI, demonstrated that μMT.apoe-/- splenocytes had increased production of IFN-γ compared to apoe-/- splenocytes (3.499 ± 1.755 vs 10.539 ± 1.869 pg/ml). Proliferation of splenocytes in response to β2-GPI was not different among the groups. These data suggest that B cells may function to modulate CD4+ T cell responses to atherogenic antigens such as β2-GPI and that in the absence of B cells, CD4+ T cells increase production of pro-inflammatory cytokines.

Aldose Reductase Expression and Activity Are Induced in Human Monocyte-Derived Macrophages by Oxidized LDL

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Aldose reductase (AR) is the rate-limiting enzyme of the polyol pathway, which utilizes excess glucose and reduces it to sorbitol and fructose. AR activity has been associated with retinopathy, nephropathy, and neuropathy in diabetic patients. Overexpression of human AR has been shown to increase atherosclerotic lesions in LDL receptor-knockout mice. Using 22 Aflahymerin gene chips (HG-U133A) we measured gene expression in human peripheral blood mononuclear cells (PBMCs) and monocytes and monocyte-derived macrophages as well as foam cells induced by native LDL, minimally modified LDL (mMDL) or oxidized LDL (oxLDL). Statistical analysis was performed using local pooled error test (LPE) and heterogenous error model (HEM). A significant increase in AR gene expression could be demonstrated in macrophages of treatment with LDL and oxLDL, however not with mMDL. Simultaneously, several genes associated with increased oxidative stress were upregulated (e.g. genes of the glutathione or thiorexin system or metallothioneins). Upregulation of AR by oxLDL was confirmed by real-time PCR. Furthermore, an increase of AR enzyme activity could be shown as measured by increased NADPH consumption. oxLDL-induced increment of AR activity was reduced when macrophages were treated with the AR inhibitor epalrestat. Weepulted that AR-expressing macrophages are involved in the pathogenesis of atherosclerosis, either by up-regulating oxidative stress genes or by facilitating cholesterol uptake.

RELATIVE LUCIFERASE ACTIVITY ±SD OF FOUR DIFFERENT SNAP NAPHTOLYPES OF HCRP

<table>
<thead>
<tr>
<th>GC</th>
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<td>5.0±0.6</td>
<td>8.5±1.6</td>
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Aldose Reductase Promotes Monocyte–Derived Macrophages by Oxidized LDL

Ashish Bhattacharjee, Srabani Pal, Lerner Rsch Institute, Cleveland Clinic, Cleveland, OH; Gerald M Feldman, Food and Drug Administration, Bethesda, MD; Martha K Cathcart; Lerner Rsch Institute, Cleveland Clinic, Cleveland, OH

NEW METHOD TO INCREASE EXPRESSION OF HCRP

Simultaneously, several genes associated with increased oxidative stress were upregulated (e.g. genes of the glutathione or thiorexin system or metallothioneins). Upregulation of AR by oxLDL was confirmed by real-time PCR. Furthermore, an increase of AR enzyme activity could be shown as measured by increased NADPH consumption. oxLDL-induced increment of AR activity was reduced when macrophages were treated with the AR inhibitor epalrestat. Weepulted that AR-expressing macrophages are involved in the pathogenesis of atherosclerosis, either by up-regulating oxidative stress genes or by facilitating cholesterol uptake.
Immunization of atherosclerosis-prone animals with malondialdehyde (MDA)-modified homologous LDL is atheroprotective. Previously, we showed that MDA-LDL immunization leads to a profound Th2 response, which in turn controls inflammation, matrix turnover, and prevents the formation of atherosclerotic plaques. These findings suggest that MDA-LDL immunization may be a promising approach for the prevention of atherosclerosis.

Immunodominant and Atheroprotective MDA-derived Epitopes

Proinflammatory Activities of C-reactive Protein and Lysophosphatidylcholine on Human Macrophages Are Selectively Suppressed by Mutual Complex Formation

Background: Both C-reactive protein (CRP) and oxidized LDL (oxLDL) trigger pro-inflammatory activities by macrophages during the process of atherosclerosis. We previously reported that CRP-LPC complex to form a complex with CRP and LPC through mutual complex formation with CRP and LPC. However, the functional relevance of the complex formation on the progression of atherosclerosis is not clear. Methods and Results: Chemosinimum immunoreactant and HPLC confirmed that lysophosphatidylcholine (LPC), a main FC-bearing component of oxLDL, binds to CRP in a calcium-dependent manner. Following AngII-infusion, primary rise of macrophage accumulation in the adventitia, consistent with an inflammatory adipokine but may not be a strong predictor of atherosclerosis, adiposity or BMI. Conclusion: We have shown that CRP and LPC are major players in the progression of atherosclerosis, and that mutual complex formation between CRP and LPC may be a promising therapeutic target for the prevention of atherosclerosis.

Risks Associated with Resistin Levels in Nondiabetic Caucasians

Resistin is an adipokine which has been linked to inflammation, insulin resistance and atherosclerosis in mice, however a similar role in humans has been debated. We have shown previously that plasma resistin levels are associated modestly with markers of inflammation and atherosclerosis but not with metabolic syndrome or insulin resistance. Human studies of resistin gene variation and these parameters are lacking. Therefore, we hypothesized that a gain of function resistin promoter SNP and associated haplotypes would be associated with resistin levels, inflammatory markers and atherosclerosis but not with lipid parameters or the metabolic syndrome. Methods: We examined the relationship of 3 resistin SNPs (–420C, –1092A and –1067C) and their associated haplotypes with plasma levels of resistin, TNF alpha and CRP, as well as adipins, NCEP-defined metabolic syndrome and atherosclerosis as measured by coronary calcium scores in Caucasians in the Study of Inherited Risk of Coronary Atherosclerosis (n = 840). Results: Plasma resistin levels were higher, dose-dependently with the G allele of the –420 polymorphism (GC 5.8 ± 2.7ng/mL, GG 6.3 ± 4.0 ng/mL and GG 7.5 ± 5.1 ng/mL, p < 0.003). There was no association with –420C>G with coronary calcium scores, BMI lipids variables or the metabolic syndrome. Estrogen reduced the 3 SNPs, that contained the G allele from the –420 variant positively associated with resistin levels (p = 0.047), CRP (p = 0.03) and TNF alpha (p = 0.02) but not the presence of the metabolic syndrome or coronary calcification. When considered alone, the –1092A>G and –1067C>C SNPs had no association with resistin levels, coronary calcium, inflammatory markers, metabolic syndrome or BMI. Conclusion: Genetic variation in resistin was not associated with coronary atherosclerosis or the metabolic syndrome. The –420C>G resistin polymorphism accounted for a small but significant amount of variation in plasma resistin levels and –420G heterozygotes were associated with CRP and TNF alpha levels. These findings are consistent with the concept that resistin is an inflammatory adipokine but may not be a strong predictor of atherosclerosis, adiposity or metabolic syndrome in humans.

Increased Presence of Macrophages Secreting Resistin in Epicardial Fat of Patients with Acute Coronary Syndrome

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Aim of the present study was to evaluate the expression of several adipocytokines in epicardial fat of patients undergoing CABG surgery for acute coronary syndrome (ACS, n = 23) as compared to that of age- and BMI-matched patients with chronic stable angina (CSA, n = 26). Patients undergoing cardiac surgery for valvular defects, but with angiographically normal coronary arteries served as reference group (n = 20). The local expression and protein secretion (24h medium) of cytokines and adipocytokines from epicardial fat biopsies were assessed by Real-Time PCR (adiponectin, leptin, resistin, visfatin, IL-6, IL-8, IL-10, CRP, MCP-1, PAI-1, MIF) and multiplexed fluorescent immunomassay (adiponectin, resistin, leptin, IL-6, PAI-1, MCP-1), respectively. Immunohistochemical stainings of epicardial fat slides were also performed to show expression of inflammatory cells (1-lymphocytes, macrophages, mast cells). The 24h medium from epicardial adipose tissue culture was also tested in experiments of endothelial
CD4+ T-Helper Cell Distributions and Associations with Atherosclerosis: Results from the Multi-Ethnic Study of Atherosclerosis (MESA)

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Introduction: CD4+ T Helper (Th) cells are key effectors of adaptive immunity and important in atherosclerosis development in mice. Less is known about their role in human atherosclerosis. Methods: We examined this association in 524 white, black, Hispanic and Chinese MESA participants. Mean age was 59 years (range 44 – 84); 56% were women. Fresh blood samples were shipped overnight to the Central Laboratory. CD4+ cells in peripheral blood mononuclear cell preparations were stimulated with ionomycin/phorbol myristate acetate. Th1 cells were defined by flow cytometry as CD4+/interferon-γ+ and Th2 cells were CD4+/interleukin-4+. Th1 and Th2 cells were expressed as percent of CD4+ cells. Total CD4+ cells were expressed as percent of lymphocytes. Results: The mean (standard deviation) for %CD4+ Th1 and %Th2 cells were 43.5% (13.4%), 14.5% (7.6%) and 0.7% (0.8%), respectively. Neither %CD4+ nor %Th1 cells were significantly correlated with inflammation markers interleukin-6 and C-reactive protein. In age, sex and ethnicity adjusted regression models, %CD4+ cells were higher with older age (1%/10yrs), female sex (4%), and white ethnicity (2.6 – 9.6% higher than others). However, %Th1 cells were lower with age (1%/10yrs) and female sex (1.2%). %CD4+ cells were associated with cytomegalovirus (CMV) serology markers (p<0.05). %Th1 cells were associated with markers of CMV and hepatitis A (both p<0.05). In stepwise regressions of coronary calcification (modeled as ln-transformed Agatston score in those with a positive score, n=237) with age, sex, ethnicity, cardiovascular disease risk factors and T cell indices, %Th1 remained significantly positively associated with degree of coronary calcification (p<0.001). Conclusions: In a multi-ethnic population of men and women, a Th1 cell distribution biased towards Th1 cell was associated with increased infectious burden and coronary calcification, suggesting a role for Th1 cells in human atherosclerosis.

Natural Killer T-Cells Influence Both Atherosclerosis and Plasma Lipid Levels in Low-Density Lipoprotein Receptor−Deficient Mice

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Natural killer T (NKT) cells are a subset of T-lymphocytes that respond to lipid antigens presented in the context of CD1 molecules and have been shown to be proatherogenic in a number of mouse models through either exogenous stimulation or genetic deficiency. To examine the effects of altering absolute NKT cell numbers in an otherwise immune competent murine model of atherosclerosis, the low density lipoprotein receptor (LDLR) deficient mouse was crossed with either the Vα14Jα18 T-cell receptor transgenic mouse possessing an increased proportion of NKT cells or the CD1d knockout mouse which lacks NKT cells. When fed a Western-type diet for 12 weeks, the Vα14Jα18LDLR−/− mice had significantly higher plasma triglyceride levels compared to CD1d−/− mice (1,364±80 vs 1,027±95 mg/dL for males, p<0.01; 573±72 vs 330±34 mg/dL for females, p<0.03; n=10−12 per group). Furthermore, the Vα14Jα18LDLR−/− mice had higher HDL cholesterol levels assessed by FPLC fractionation (96±8 vs 139±12 mg/dL for males, p<0.01; 94±10 vs 115±15 mg/dL for females, p<0.05). The Vα14Jα18LDLR−/− mice had larger atherosclerotic lesions than the CD1d−/−LDLR−/− mice in both the inanominate artery (46,680±16,923 vs 19,074±5,737 μm² for males, p<0.01; 43,248±15,906 vs 5,414±3,183 μm² for females, p<0.02) and ascending aortic arch (63,841±8,817 vs 36,008±2,866 μm² for males, p<0.02; 74,033±16,585 vs 32,924±3,425 μm² for females, p<0.01). No changes in aortic root atherosclerosis were noted, though earlier time points are currently being examined. In sum, NKT cells have the potential to subtly impact atherogenesis by affecting systemic lipid levels in addition to any local actions in the atherosclerotic plaque.

Overexpression of Glutathione Peroxidase 4 Reduces Atherosclerosis in Apolipoprotein E−Deficient Mice

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Accumulation of oxidized lipids in the arterial wall is believed to give rise to atherosclerosis. Glutathione peroxidase 4 (GPx4) is a peroxide scavenger that removes oxidative modifications from lipids, e.g., free fatty acids, cholesterol and phospholipids. The primary goal of this study is to assess the effect of overexpressing or downregulating GPx4 on atherosclerosis in apoE−/− and apoE−/− control mice. Our data demonstrated that overexpression of GPx4 in the aortic and the aortic sinus of the ApoE−/− mice overexpressing GPx4 (hPGx4Tg/ApoE−/−) were significantly smaller than those of the ApoE−/− control mice. Almost all of the ApoE−/− control mice at 4 – 5 months of age developed both early stages of atherosclerotic lesions (e.g., foam cells and free lipids) and advanced lesions (e.g., fibrous caps and acellular areas) in the aortic sinus. In contrast, only about two thirds of the hPGx4Tg/ApoE−/− mice developed atherosclerotic lesions in the aortic sinus and the aortic sinus of the ApoE−/− mice overexpressing GPx4 (hPGx4Tg/ApoE−/−) were significantly smaller than those of the ApoE−/− control mice. Almost all of the ApoE−/− control mice at 4 – 5 months of age developed both early stages of atherosclerotic lesions (e.g., foam cells and free lipids) and advanced lesions (e.g., fibrous caps and acellular areas) in the aortic sinus. In contrast, only about two thirds of the hPGx4Tg/ApoE−/− mice developed atherosclerotic lesions in the aortic sinus and the aortic sinus of the ApoE−/− mice overexpressing GPx4 (hPGx4Tg/ApoE−/−) were significantly smaller than those of the ApoE−/− control mice. Almost all of the ApoE−/− control mice at 4 – 5 months of age developed both early stages of atherosclerotic lesions (e.g., foam cells and free lipids) and advanced lesions (e.g., fibrous caps and acellular areas) in the aortic sinus.
to oxidized lipids are the mechanisms by which GP4X inhibits atherosclerosis. Unexpectedly, heterogeneous mutation to GP4X did not increase atherosclerotic lesions and oxidized lipids in ApoE−/− mice.

Structural and Lipid-Binding Properties

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Human apolipoprotein A-V (apoA-V) is a potent modulator of plasma triacylglycerol (TG) levels. To probe different regions of this 343 amino acid protein, 4 single Trp apoA-V variants were prepared. The variant with a Trp at position 325, distal to the tetra-proline sequence at residues 295–296, displayed an 8 nm blue shift in wavelength of maximum fluorescence emission upon lipid association. To evaluate the structural and functional role of this 51-residue C-terminal segment, a truncated apoA-V, comprising amino acids 1–292, was generated. Far UV circular dichroism spectra of full-length apoA-V and apoA-V(−1100) were similar with ~50% alpha helix content. In guanidine HCl denaturation experiments, both full-length and truncated apoA-V yielded biphasic profiles consistent with the presence of two structural domains. The denaturation profile of the lower stability component, but not the higher stability component, was affected by the truncation. In fluorescent dye binding experiments, apoA-V(−1100) contained fewer solvent exposed hydrophobic sites than full-length apoA-V. Truncated apoA-V displayed an attenuated ability to solubilize DMPC phospholipid vesicles compared to full-length apoA-V yet it bound to a triolein/water interface with faster kinetics. Taken together, the data suggest that the conformational stability of apoA-V(−1100) and the tetra-proline rich C-terminal sequence is due to the presence of a higher stability component that is necessary for apoA-V to adopt a folded protein structure yet functions to modulate apoA-V lipid binding activity and thereby, may be relevant to the mechanism by where it influences plasma TG levels.
than HDL₃. Human apoA-I is a major determinant of the biphasic distribution of HDL. ApoA-I is made up of 10 repeating amphipathic α-helices (11 or 22 a.a.) interrupted in most cases by proline residues, except between helices 7 and 8. We generated apoA-I mutants in which the interhelical sequence (IHS) between helices 7/8 (7 residues) was substituted with each of the remaining IHS found in huA-I. The mutant and wild-type (wt) proteins did not significantly differ in their secondary and functional properties examined. In addition, the recombinant huA-I and HDL subfractions, we observed that wt huA-I has a near equal affinity for HDL₃ (1.42 μM) and HDL₂ (1.63 μM). In contrast, huA-I mutants with IRS3/4 or IRS9/10 replacing IRS7/8 preferentially associated with HDL₂ and displayed higher Kₘ association values for HDL₃ (0.49 μM and 0.27 μM respectively) versus HDL₂ (1.12 μM and 4.6 μM respectively). The huA-I mutant in which IRS5/4 replaced IRS7/8 preferentially associated with HDL₃ and displayed a higher Kₘ association constant for HDL₃ (0.63 μM) versus HDL₂ (5.4 μM). To examine if the mutant huA-I proteins generate nascent HDL particle of different densities, we stained nascent HDL rat hepatocytes expressing exogenous huA-I proteins with TEM. We found comparable levels of Kₘ protein and species-specific huA-I antibodies, we found that wt huA-I forms two major nascent HDL particles with peaks at 1.0901 ± 0.001 μg/ml and 1.1557 ± 0.001 μg/ml. In contrast, huA-I (IRS7/8/IRS3/4) generates a single nascent HDL between 1.1037 ± 0.001 μg/ml and 1.1191 ± 0.001 μg/ml. The peak of the nascent HDL formed by huA-I (IRS7/3/IRS4/5) mutant is 1.3692 ± 0.001 μg/ml, but also appears to have a shoulder at a lower density. The distribution of the endogenous rat apoA-I on the nascent HDL followed that of the huA-I protein in each case. In conclusion, the IHS placed between helices 7 and 8 in huA-I can influence affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL₂ and HDL₃, but also appears to have a shoulder at a lower density. The distribution of the endogenous rat apoA-I on the nascent HDL followed that of the huA-I protein in each case. In conclusion, the IHS placed between helices 7 and 8 in huA-I can influence affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclasses, we observed that wt huA-I has a near equal affinity for HDL subclass.
ESR1 gene region appears to influence measures of plasma TG metabolism in a gender- and age-specific manner.

Lecithin-Cholesterol Acyltransferase Can Rescue the Abnormal Phenotype Produced by the Natural Apoipoprotein A-I Mutations (Leu141Arg) Pisa and (Leu159Arg) FIN

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Objective: To explain the etiology and find mode of therapy of low HDL observed in humans harboring two natural apoA-I mutations, (Leu141Arg) Pisa and (Leu159Arg) FIN. Methods: We have generated recombinant adenoviruses expressing apoA-I(Leu141Arg) Pisa and apoA-I(Leu159Arg) FIN and studied the properties of the mutant proteins in vitro and in vivo. Both mutants were secreted efficiently from cells but had diminished capacity to activate LCAT in vitro. Adenovirus-mediated gene transfer of either of the two mutants in apoA-I deficient mice resulted in greatly decreased total plasma cholesterol, apoA-I, high density lipoprotein (HDL) cholesterol levels, cholesteryl ester to total cholesterol ratio (CE/TC), preponderance of preβ1-HDL, and small size apoA-I HDL particles and generated only few spherical HDL particles, as compared to mice expressing WT apoA-I. Simultaneous treatment of the mice with adenosine inhibitors expressing either of the two mutants and human LCAT, normalized the plasma apoA-I, HDL cholesterol levels, and the CE/TC ratio, restored normal preβ1- and α-HDL subpopulations and generated spherical HDL. Conclusion: The study establishes that apoA-I(Leu141Arg) Pisa and apoA-I(Leu159Arg) FIN inhibit an early step in the biogenesis of HDL due to inefficient esterification of the cholesterol of the preβ1-HDL particles by the endogenous LCAT. Both defects can be corrected by treatment with LCAT.

Identification and Molecular Characterization of New PCSK9 Missense Mutations Associated with Familial Hypercholesterolemia

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Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) is a bona fide inhibitor of the LDL-receptor. In humans, PCSK9 gain of function mutations are associated with FH, whereas mutations inactivating PCSK9 are associated with reduced plasma LDL and cardiovascular events. Characterization of the naturally occurring mutations reported to date has provided some insights into PCSK9 mechanisms of action but it has not been possible to distinguish the phenotypic effect of some gain of function from some loss of function mutations based on their autocalyptic cleavage and secretion pattern. In the present study, we analysed the PCSK9 exons and intronic junctions of FH patients found to be non LDL-receptor or apoipoprotein B100 mutation carriers. The previously reported S127R French mutation was found in a South-African family, whereas new heterozygous missense mutations D129G and A166E were found in two families from New Zealand. Except for the A166E, these mutations modify a highly conserved residue. Segregation with the FH phenotype was also incomplete in the A166E family. PCSK9 overexpression studies in HuH7 hepatoma cells shows that both S127R and D129G missense PCSK9 mutants have 75% reduced autocalyptic activity compared to wild type, whereas the A166E mutant is processed normally. The S127R and D129G mutants were not secreted in the culture media, unlike both the A166E mutant and wild type PCSK9. Cellular LDL binding was decreased by 25–30% in cells overexpressing S127R and D129G mutants compared with those overexpressing wild-type PCSK9. Overexpression of the A166E mutant resulted in a non-significant 10% decrease in cellular LDL binding vs. wild-type. The cell surface LDL receptor levels paralleled those of cellular LDL binding. Our study indicates that (1) the region within the prodomain of PCSK9 encompassing the S127 and D129 residues is critical for PCSK9 autocalyptic activity and secretion, and that (2) two non-secreted naturally occurring mutants of PCSK9 inhibit LDL receptor expression and activity, suggesting that PCSK9 mediated inhibition of the LDL receptor in the liver occurs intracellularly.

Antisense Inhibition of Apolipoprotein B-100 Significantly Reduces LDL Cholesterol and Glucose Levels in Ob/Ob Mice

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In previous studies, we demonstrated that intraperitoneal (i.p.) administration of a mouse-specific apolipoprotein B-100 (apoB-100) antisense oligonucleotide (ASO), ISIS 147764, produced dose- and time-dependent reductions in hepatic apoB mRNA and protein and serum apoB-100, total cholesterol and triglycerides in apoE deficient mice. These in vivo antisense effects have been confirmed in multiple animal models and most importantly, in humans during Phase 1 and 2 clinical trials. As a large number of individuals with diabetes also suffer from dyslipidemia and coronary artery disease, we administered ISIS 147764 to ob/ob mice on a high fat/cholesterol diet in order to determine the efficacy of inhibiting apoB-100 in insulin-resistant mice. Treatment of these animals with 50 mg/kg/wk of ISIS 147764 for 6 weeks resulted in a 65% reduction in hepatic apoB mRNA with a commensurate reductions in total cholesterol (34%), VLDL-C (61%), LDL-C (55%) and serum triglycerides (21%). Serum glucose levels were also reduced by 33% when compared to control apoB-100 mice. As observed in other murine hyperlipidemia models, reductions in serum apoB-100 did not cause hepatic steatosis as determined by Oil Red 0 staining of livers and quantitation of liver triglyceride levels. Furthermore, ASO administration in these mice was well tolerated. These results and the small size from the lab indicate the clinical utility of e-122

The N-Terminal 50 Amino Acid Residues of the β domain of Apolipoprotein B Induce Instability in Lipoprotein Particle Assembly

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Apolipoprotein B (apoB)-100, essentially the only protein component of the atherogenic LDL, has a pentapartite structure, NH2-β1-β2-β3-β4-β5-COOH. The β domains contain multiple amphipathic β strands and the α domains contain multiple amphipathic α helices. The β3 domain, like lamprey lipovitellin (LV), is a globular composite of α-helices and β-sheets. We previously proposed that the apoB particle assembly is initiated when the β1 domain, comprising of the first 1000 amino acid residues of apoB1000 (designated apoB1000), folds into a LV-like lipid pocket to form the apoB “lipid pocket”. We demonstrated that in stable transfomers of MCA-RH7777 cells, apoB1000 is secreted as a stable monodisperse phospholipid-rich particle. We also showed that apoB1200, containing 200 residues of the β1 domain, of apoB is secreted predominantly as a lipid-poor particle with only a fraction of the protein as a relatively lipid-rich particle. To map the effect of each domain of amphipathic β strands within the N-terminal region of the β1 domain, on the relative levels of secretion of large versus small particles, we made sequential truncations of region between apoB1200 and apoB1000 to produce apoB1050, apoB1100, and apoB1150. Characterization of the secreted particles by metabolic labeling of stable transformers of MCA-RH7777 cells with [3H]glycerol and their isolation by non-denaturing gradient gel electrophoresis, demonstrated that the presence of only 50 amino acid residues of the N-terminal region of the β1 domain causes instability in the particle. Thus, in contrast to apoB1000, apoB1050 was secreted in two forms, a large lipidated particle and a small lipid-poor inclusion. Inclusion of residues 1050 to 1100 resulted in further destabilization in the particle. ApoB1100 appeared to form at least four particles, suggesting that the domain between residues1050 and 1100 might be more destabilizing than the previous 50 residues. ApoB1150 formed particles that were similar to those formed by apoB1050, suggesting that the domain between residues 1100 and 1150 might partially restore particle stability. In conclusion, our results suggest that not all the sequences in the N-terminal 200 residues of the β1 domain are equal in their effects on particle stability.

ABC1 and SR-BI Are Significant Contributors to in Vivo Reverse Cholesterol Transport from Macrophages

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BACKGROUND: Cholesterol transporters, ABCA1, ABCG1 and SR-BI, are key mediators of cholesterol efflux to apoA-I and HDL, which represents the first step of reverse cholesterol transport (RCT) in vivo. However, the individual contributions of each transporter to in vivo RCT, defined here as transport from macrophages to liver and further into bile have not been thoroughly measured. Here we first measured the in vivo RCT from cholesterol labeled macrophages with either ABCA1 or SR-BI deficiency. METHODS AND RESULTS: Bone marrow derived macrophages from abca1 (-/-) or sr-bi (-/-) or control mice were labeled with 3H-cholesterol or-ACLDL or 3H-cholesterol-LDL and injected into control mice. After injection, return of 3H-cholesterol from labeled abc1 (-/-) or sr-bi (-/-) macrophages to serum, liver, bile and feces, was measured. ABCA1 deficiency in macrophages reduced cholesterol return from macrophages which labeled with [3H]cholesterol-ACLDL by up to 50% from control mice, dependent on the readout (plasma, liver or bile radioactivity). SR-BI deficiency only reduced cholesterol return from LDL labeled macrophages, but not from acLDL labeled macrophages, consistent with the role of SR-BI in efflux to HDL and not to apoA-I. CONCLUSION: These results indicate that both ABCA1 and SR-BI play significant roles in efflux and in vivo RCT, consistent with their protective functions against development of atherosclerotic lesions.
Diurnal Transcriptional Regulation of Microsomal Triglyceride Transfer Protein and Plasma Lipid Levels

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Several cardiovascular diseases such as atherosclerosis and coronary heart disease exhibit circadian variations. High plasma lipid levels are risk factors for these diseases and plasma lipids also show diurnal variations. Our aim was to identify molecular mechanisms controlling diurnal variations in plasma lipid levels in rats and mice maintained in a 12-h photoperiod with free access to a low-cholesterol, low-fat diet. Plasma triglyceride and cholesterol levels were high in the dark than in the light phase in these animals. These variations were mainly due to changes in apolipoproteins, as HDL levels did not show circadian rhythm. Intestinal lipoprotein production studies revealed that the absorbance of [1H]oleic acid or [1H]cholesterol was high at 12:00 h. These observations were supported using in situ rat intestinal loops indicating that intestinal lipoprotein production, independent of gastric emptying, shows diurnal variation. Since MTP is critical for apolipoprotein biosynthesis, we investigated intestinal MTP activity and protein levels at different times in rats and mice. Both MTP activity and protein showed significant variations. Over time, changes were observed in the expression of MTP mRNA. The in vitro synthesis of MTP by enteroxymes isolated at 24:00 h was higher than those isolated at 12:00 h. To assess whether diurnal variations in MTP synthesis occurred at the transcriptional level, we measured steady state mRNA levels. MTP mRNA levels showed diurnal variations and were higher in the light phase than in the dark phase. No changes were observed in CD36 mRNA levels. In conclusion, our data show that all MTP possess similar secondary and tertiary structures. In addition to their structural similarities, MTP homologs were found to share several biochemical properties. Expression of candidate proteins from each group as FLAG chimeras showed that all proteins were associated with protein disulfide isomerase in the endoplasmic reticulum as well as in the Golgi, and supported assembly and secretion of apolipoproteins. In vitro lipid transfer assays revealed that overexpression of MTP in nonproteasomal apoB turnover.

Acquisition of Triacylglycerol Transfer Activity by Microsomal Triglyceride Transfer Protein During Evolution

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Microsomal triglyceride (TG) transfer protein (MTP) is essential for the assembly of neutral lipids into lipoprotein particles. We previously reported that the Drosophila MTP does not transfer TG but transfers phospholipids. In contrast, human MTP transfers both lipids. To explore the acquisition of TG transfer activity by MTP during evolution, we obtained TG transfer activity from sequences of birds, amphibians and insects that were homologous to human MTP. Sequence comparison revealed a specific sequence. Using this sequence we identified homologous proteins in nematodes. Based on phylogenetic analysis, we divided these proteins into four groups (mammals, mammals, fish, insects, and nematodes). Structural analysis demonstrated that all MTP possess similar secondary and tertiary structures. In addition to their structural similarities, MTP homologs were found to share several biochemical properties. Expression of candidate proteins from each group as FLAG chimeras showed that all proteins were associated with protein disulfide isomerase in the endoplasmic reticulum as well as in the Golgi, and supported assembly and secretion of apolipoproteins. In vitro lipid transfer assays revealed that overexpression of MTP in nonproteasomal apoB turnover.

Overexpression of ER60 Induces Apolipoprotein B Degradation and the Secretion of an 80 kDa Fragment in HepG2 and McA-RH777 Cells: Role of ER60 in Nonproteasomal apoB Turnover

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We have previously shown that overexpression of ER60 in HepG2 cells increases intracellular degradation of apolipoprotein B (apoB) (Qiu et al., Biochemistry 2004, 43:4819–4831). Here we report the identification of an ER60-induced proteolytic fragment of apoB that is secreted following ER60 overexpression. ApoB stability was found to be significantly reduced with ER60 overexpression in McA-RH777 and HepG2 cells transiently transfected with human apoB48. Interestingly, an 80 kDa fragment of apoB was detected and found to be predominantly secreted into the media of both McA-RH777 and HepG2 cells following ER60 overexpression. The generation and secretion of the 80 kDa apoB fragment was detected in the presence or absence of MTP based on MTP transfusions study and by mttp knockdown. Importantly, generation of the 80 kDa apoB fragment was only observed with ER60 overexpression and not other ER chaperones such as GRP78, which has been shown to promote proteasomal apoB degradation. The secreted 80 kDa apoB fragment was insensitive to the inhibition of ALLN, lactacystin, MG132 or PCMB, which previously showed partial inhibitory activity toward a 50 kDa apoB fragment generated in permeabilized HepG2 cells overexpressing ER60. Using [35S]

Adenoviral Overexpression of Hormone-sensitive Lipase and Adipose Triglyceride Lipase Promotes Fatty Acid Oxidation and Reduces Hepatic Steatosis Without Affecting VLDL Secretion in ob/ob Mice

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Hepatic steatosis is often associated with the insulin-resistant state. We demonstrated that hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), two enzymes critical for lipolysis in adipose tissues, could mobilize hepatic triglycerides (TG) in vivo and in vitro without adverse effects. Adenoviral overexpression of HSL and ATGL in ob/ob mice decreased lipid droplet size and reduced liver TG mass by 50%–60% but had no effects on fasting plasma TG levels or TG and apoB secretion rates. There was no apparent effect on the expression of genes involved in fatty acid (FA) uptake (CPT3) and synthesis (FAS, PPAR⁻γ), triacylglycerol and cholesterol synthesis. Hepatic steatosis was reversed in ATGL⁻/⁻ and TAM也不例外，which were observed in the absence of intraperitoneal or intraduodenal triglycerides. Our results demonstrate that ATGL overexpression increased liver cellular diacylglycerol. These observations are consistent with the respective substrate specificities of these two enzymes. HSL overexpression also increased TG secretion and decreased intracellular apoB degradation resulting in an increase in apoB secretion, an effect seen in vivo. In summary, hepatic overexpression of HSL or ATGL promotes FA oxidation and reduces hepatic steatosis without affecting VLDL secretion; the decrement in vivo and in vitro effects of HSL overexpression on VLDL secretion possibly result from differences in cellular machinery available in vivo or in vitro. Overall, this report identifies HSL and ATGL as potential therapeutic targets for ameliorating hepatic steatosis associated with insulin resistance and obesity.

Postprandial Lipid Effects of a 1,3-Diacylglycerol Oil Versus Triacylglycerol Oil in an Insulin-Resistant Population

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Background: Postprandial (PP) hypertriglyceridemia is common in individuals with insulin resistance with or without concomitant type 2 diabetes mellitus, and has been associated with the presence of both coronary and carotid artery atherosclerosis. Studies in rodents and humans have shown that fatty acids from dietary DAG are not efficiently incorporated into chylomicrons after absorption from the intestinal lumen. Some studies with diets enriched in 1,3-diacylglycerol (DAG) oil have shown reduced PP triglyceride (TG) levels compared with diets containing triacylglycerol (TAG) oil. It is important to identify new diet approaches that would benefit patients with insulin resistance. Methods: We enrolled 25 insulin resistant (HOMA >2.5)-non diabetic subjects in a double-blind, randomized crossover trial to test acute and chronic effects of a DAG diet compared with a TAG diet. Subjects received DAG oil or TAG oil, in food products, for five weeks and were then crossed-over to the other oil after one-a week washout groups (three week diet period, one week washout). PP fatty acid tests, one with each oil. Results: PPG areas under the curve (AUC) over 8 hours (mean ± SD 2007 ATVB Poster Presentations e-123

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Effects of P4P on Lipoprotein levels

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Measurement of Cholesterol Efflux and Global Reverse Cholesterol Transport Rates in Vivo with Stable Isotopes

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RCT, the transport of cholesterol (C) from peripheral tissues and out of the body, is the only route for reducing C burden in tissues, including vascular wall macrophages. RCT is the leading explanation for the cardio-protective effects of HDL. Accordingly, interventions that stimulate flux through the RCT pathway represent a therapeutic target. There had not previously been a method for measuring the first arm of the pathway (efflux of C from tissues) for RCT. We have developed an in vivo method for quantifying efflux rates and flux through the global RCT pathway. The method involves non-radioactive (stable) isotopes, is non-invasive, and can be used in humans. Efflux is measured by the isotope dilution principle, through a constant infusion of labeled C into free C pool, followed by an intravenous injection of a potent RCT activator. Following administration, plasma HDL is isolated and cholesterol efflux is measured. Replicate studies were performed in healthy human volunteers. RCT flux through the RCT pathway was correlated with higher HDL-C and lower LDL-C.

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EP 80318: A Novel CD36 Ligand with Hypcholesterolemic and Anti-Atherosclerotic Properties in Apo-a-Mice

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Introduction. Macrophage scavenger receptor CD36 is known to mediate internalization of oxidized lipoproteins at the level of the intima in arteries with the formation of foam cells as the first step of atherosclerotic lesion development. We have previously reported that EP 80318, a growth hormone-releasing analogue (GHRH-analog) with selective CD36 binding properties exerts significant CD36-dependent anti-atherosclerotic effect in mice. Whether this anti-atherosclerotic effect is shared by other members of GHRP family is not known. A new GHRP analog EP 80318 (Abz-D-Met-D-Phe-D-Leu) with specific binding affinity towards CD36 at 25 μM was used to document anti-atherosclerotic properties in apo-a-mice used as atherosclerotic murine model. Methods. Apo-a-mice fed a HFD for 4 weeks old were administered a daily s.c. dose of either of two selective CD36 ligands, EP 80318 or EP 80317 (500 μg/kg) or 0.9% NaCl, from 6 to 18 weeks of age (n = 6–9/group). Blood was withdrawn from the subclavian vein and aortas were isolated from the aortic arch to the iliac bifurcation and cut longitudinally under stereomicroscope. Neutral lipids were colored by oil red o staining and the percentage of atherosclerotic lesions was determined by morphometric analysis. Plasma lipids were quantified by enzymatic methods. Results. A chronic treatment with EP 80318 or EP 80317 reduced the percentage of total aortic lesions by 30% (p < 0.01) and 41% (p < 0.01) compared to 0.9% NaCl, respectively. This effect was associated with a hypocholesterolemia, as shown by a reduction of 31% (p < 0.05) of total plasma cholesterol in mice treated with EP 80318 and 26 % (p < 0.05) for mice treated with EP 80317 (22.6 ± 2.2 mmol/L in controls, 15.5 ± 1.3 mmol/L and 16.8 ± 2.1 mmol/L in mice treated with EP 80318 and EP 80317, respectively). In contrast, neither triglycerides, nor HDL cholesterol plasma concentrations were modulated by either one of the treatments. Conclusion. Our results support the potential application of GHRP derivatives targeting CD36 for the prevention of atherosclerotic lesions development.

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Antibody Response to Several Different Autoantibodies Is Strongly Associated with Acute Coronary Syndromes

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After the last several years, the idea that immune processes play a key role in atherosclerosis and its complications, acute coronary syndromes (ACS), has received attention. We assessed...
the hypothesis that different auto-antibodies might be involved in the plaque formation and instability and lead to appearance of ACS. Methods and Results: The study included 211 participants; of whom 111 were patients with ACS (60.8 ± 4.57 years of age, 60% males) and 100 were aged matched controls with no known coronary artery diseases - Blood donors. Patients with previous infection, surgery, trauma or concomitant rheumatological diseases were excluded from the study. Blood was sampled, frozen on - 40°C and sent on day 3 to Immunosciences Lab. Inc (USA) for analysis. All traditional risk factors were noted. Antibodies (IgG) against endothelial cells, oxidized LDL, beta 2 glycoprotein 1, plasmin, platelet membrane glycoprotein and bacterial HSP 70 were determined as well as levels of interleukin 6, high sensitivity CRP, homocysteine, lipoprotein (a) and platelet membrane glycoprotein. Our data indicated: At adequate cholesterol concentrations of examined antibodies in patients with ACS. The levels of circulating antibodies and novel risk factors were significantly higher in patients. Linear regression confirmed that immune - inflammatory activation and thrombosis might be due to autoimmune reactions. In connection with the atherosclerosis for several auto-antibodies appears to be very sensitive and a new marker of ACS. It causal involvement and its diagnostic role in ACS and atherothrombosis itself deserve further study.

Acetaldehyde Increases Endothelial Cell Chemokine mRNA Expression

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A biphasic effect of alcohol on the incidence of cardiovascular disease has been documented, where moderate consumption of alcohol exerts a protective effect while chronic alcohol abuse and/or binge drinking are associated with a higher incidence of cardiovascular disease. The subtle balance between alcohol and its primary metabolite, acetaldehyde, has a crucial impact on the physiological consequences of alcohol consumption. Inflammatory mediators have been shown to play an important role in the progression and destabilization of the atherosclerotic plaque. We hypothesize that binge alcohol consumption exacerbates cardiovascular disease, in part by elevated concentrations of its primary metabolite, acetaldehyde, causing an increase in chemokine expression. Methods: Human umbilical venous endothelial cells (HUVECs), passages 3-5, were treated with acetaldelyde (5–100 μM) for 6h. HUVEC MCP-1, TNFα and IL-6 mRNA were detected by qRT-PCR. Our data indicated: At adequate cholesterol concentrations of antibodies in patients with ACS. The levels of circulating antibodies and novel risk factors were significantly higher in patients. Linear regression confirmed that immune - inflammatory activation and thrombosis might be due to autoimmune reactions. In connection with the atherosclerosis for several auto-antibodies appears to be very sensitive and a new marker of ACS. It causal involvement and its diagnostic role in ACS and atherothrombosis itself deserve further study.

Deficiency of AT1a Receptors Profoundly Reduces Sidestream Cigarette Smoke–Augmented Atherosclerosis in LDL Receptor -/- Mice

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Objective: The renin-angiotensin system has been implicated in the development of the atherosclerotic vascular diseases through mechanisms that are independent of blood pressure. Administration of angiotensin type 1a receptor antagonists or a deficiency of AT 1a receptors, markedly reduces hypercholesterolemia-induced atherosclerosis. We have previously shown that inhalation exposure to cigarette smoke increases atherosclerotic lesion formation in hypercholesterolemic mice. To determine if AT1a plays a role in sidestream cigarette smoke-augmented atherosclerosis, we examined the formation of aortic lesions in AT1a receptor -/- mice. Methods and Results: AT1a receptor +/- and -/- mice in an LDL-/ background were maintained on a chow diet for 12 weeks. Control groups were exposed to ambient air. Significant increases in urinary cotinine and lung tissue CYP 1A1 levels in exposed animals confirmed their effective exposure to cigarette smoke. Concentrations of oxidative stress biomarker 8-OHdG were significantly increased in the urine of smoke-exposed mice. Plasma cholesterol concentrations and lipoprotein cholesterol distributions were not affected significantly by either the AT1a receptor genotype or the exposure to sidestream cigarette smoke. The exposure to sidestream cigarette significantly increased the size of atherosclerotic lesions in AT1a receptor -/- mice. Deficiency of AT1a receptors produced a dramatic reduction in the size of atherosclerotic lesions. Conclusions: These results are consistent with AT1a receptors having a profound effect on atherogenesis promoted by both hypercholesterolemia and sidestream cigarette smoke.

id3 Deficiency Increases Atherosclerosis in Apoe Knockout Mice

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Background: Inhibitor of differentiation-3 (Id3) has recently emerged as an important regulator of cell response to injury, although its role in known. To examine its role in atherosclerosis, we first tested whether id3 was increased in atherosclerotic lesions in vivo. To examine this, we crossed id3-/- mice to atherosclerosis prone Apoe-/- mice to produce id3-/-/Apoe-/- double knockout mice (DKO). Methods: Beginning at eight weeks of age, male Apoe-/- or DKO mice were fed either standard chow or Western diet for 16 or 32 weeks. Mice were euthanized by ketamine/xylazine overdose and perfusion-fixed with saline prior to removal of the whole aorta. Aortic arches were paraffin embedded, sectioned in 5 μm intervals from the cusp to the bifurcation of the brachiocephalic artery and stained using Russell's modified Movat method. Descending aortas were stained for lipid content using Sudan IV, pinned open longitudinally and analyzed by enface. Lesion sizes from both the arch and the descending aorta were quantified using ImagePro software. The two-tailed Mann-Whitney U test was used for analysis and p values < 0.05 were judged to be significant. Results: Absence of Id3 in Apoe-/- mice had no effect on lesion formation in the arch or aorta, but with a trend towards reduction at 32 weeks in the aorta. Conclusions: These results support that id3 has a protective effect on the aortic lesion. Further studies will be needed to determine the mechanism for the observed difference in atherosclerotic lesion formation.

Absence of CD36 and SR-A/Ii Protects Against Atherosclerosis in Apoe Knockout Mice: Protection Is Equivalent to Absence of CD36 Alone

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Objective: The role of scavenger receptors in atherogenesis has become controversial as a result of conflicting results and a recent proposal that suggested that scavenger receptor absence would enhance the pro-inflammatory, pro-ooxidic and pro-dyslipidemic activities. The purpose of this study was to determine the effect of combined absence of scavenger receptors CD36 and SR-A/Ii on atherosclerosis lesion development in the apoe0 model. Methods: We created background matched strains of apoE0/apoE0, CD36o/CD36o and CD36o/SRAo/ apoEo that were greater than 95% C57Bl/6. These mice were fed a Western diet at 4 weeks of age for 16 weeks. Results: In DKO mice, there was a greater than 50% reduction in lesion formation compared to ApoE0 controls. There was a 61% and 74% decrease in aortic tree lesion area in CD36oApoe0 males and females, respectively, compared with apoE0 controls. Combined absence of CD36 and SRA provided no further protection in either gender. Absence of SRA was protective (32% decrease in lesion) in female mice. Importantly, combined absence of CD36 and SRA did not result in increased atherosclerosis as would be predicted by a recent hypothesis.

Sphinogine 1-Phosphate Reduces Oxidized LDL–Mediated Upregulation of Macrophage CD36

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CD36 is highly expressed on macrophages where it recognizes oxidized lipids that are present in apoptotic cells and in oxidized LDL (oxLDL). CD36 mediates lipid accumulation and macrophage foam cell formation associated with atherosclerosis. Macrophages from C57Bl/6J (B6) mice were isolated by peritoneal lavage, and incubated with oxLDL, (50μg/ml), S1P (500μM) or oxLDL plus S1P for 4, 12 and 24h. CD36 mRNA and protein levels were measured. We observed that the timeframe of macrophage CD36 mRNA expression is significantly delayed compared to macrophage mRNA expression in B6 mice. CD36 mRNA or protein after 4h or 12 treatments with oxLDL and/or S1P. However, incubation of B6 macrophages for 24h with oxLDL showed a significant induction of CD36 mRNA and protein. Incubation of B6 macrophages with S1P for 24h showed approximately a 50% reduction in basal CD36 mRNA and protein. Additionally, S1P inhibited oxLDL-mediated upregulation of CD36. To investigate the mechanisms for the S1P regulation of CD36 expression, mRNA and protein stability assays were performed using S1P, actinomycin D and cycloheximide. No significant differences were found, suggesting that S1P does not affect CD36 mRNA or protein stability. 12/15LO products have been shown to regulate PPARγ, which is in turn regulated by CD36. We evaluated the involvement of 12/15LO gene expression using by comparing expression in B6 mice and 12/15LO-/ mice, which we found no differences in macrophage CD36 expression. Further, we did not observe changes in 12/15LO activity in B6 macrophages treated with S1P. However, we observed a significant induction of CD36 expression when B6 macrophages were co-incubated with S1P and 12/15LO products. Sphingosine 1-phosphate promotes macrophage survival and regulates CD36 expression, both of which are critical for regulating macrophage foam cell formation and apoptotic cell uptake in the vessel wall. The increase in Akt phosphorylation is consistent with regulation of CD36 expression, thus, we anticipate that Akt is directly involved in the S1P-mediated regulation of CD36. S1P may be a novel regulator of macrophage function in atherosclerosis by promoting macrophage cell survival and controlling macrophage apoptotic cell uptake and foam cell formation by CD36.

Contribution of Myocardial Small Arteries in the Development of Human Coronary Atherosclerosis

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Background: The solution of HDI problem is hidden in its prevention. Do we know all risk factors? Aim. The purpose of the study was to look for HDI risk factors in myocardial microcirculatory bed. Material and methods. The study was carried out on heart of 80 people (42 healthy in age of 24 – 87 years died of the violent reasons and 38 died from the first acute myocardial infarction in age of 50 – 69 years). Methods of autopsy, morphometry, x-ray micrometric angiography, histology, hemodynamics and cytochemistry have been used. The statistics have been used. With developed method the volume density of intramyocardial arterial bed of the left ventricle wall (Vvart) on histological slides was measured. The stage and intensity of atherosclerotic lesions in main coronary arteries, the degree of stenosis were evaluated. Results. In hearts prepared for angiography the index Vart exceeded in 9 times the value of a similar index for the hearts not subjected to injection. In average the value of Vart. of hearts with a myocardial infarction was 28 %
below of healthy hearts index. There was a significant variation of the value of Vvart. both among healthy people and patients. The distribution of indices Vvart. was different. In group of healthy young and middle-aged persons the indices Vvart. were distributed with comparatively regular intervals between extremes. The Vvart. of elderly and senile healthy persons was piled up mainly at the top borders of the scale. In group of myocardial infarction the indices Vvart. were accumulated near to bottom border of scale. Significant negative correlation between value of Vvart. and coronary atherosclerosis intensity was found out both in healthy people and at died of myocardial infarction. Conclusion. Due to quantification of morphological changes and anatomical features of heart an earlier unknown risk factor of IHD was disclosed. It has two negative influences. At low value of volume density of heart intramural arterial bed both intramyocardial blood supply suffers and development of coronary atherosclerosis is accelerated. Combined using of radiological (MRI, CT, ultrasound) and developed morphological methods could reveal correlation between results of two methods. Afterwards with only non-invasive methods indicated risk factor of IHD in once lifetime can be exposed.

Dietary 7-ketocholesterol Does Not Alter the Extent of Atherosclerosis in Male LDL Receptor–Deficient Mice Despite a Decrease in Hepatic Paraoxonase-1 Expression

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Dietary oxysterols have been implicated in the pathogenesis of atherosclerosis. Diets enriched in oxysterols decrease activity of paraoxonase-1 (PON-1), a circulating atheroprotective protein, and lead to enhanced atherosclerosis despite decreased plasma cholesterol levels. Whether individual oxysterols have similar effects is unknown. The most abundant dietary oxysterol is 7-ketocholesterol (7KC). Culturing AML12 mouse hepatocytes for 24 hours with 10μM of 7KC, but not unoxoidized cholesterol or other oxysterols, decreased PON-1 and apoA-1 production and increased the inflammatory protein, serum amyloid A (SAA). These changes were mediated by NF-κB activation and might decrease the atheroprotective effects of HDL. We hypothesized that adding 7KC to an atherogenic diet would lead to similar changes in the hepatic expression of these proteins in vivo, and increase atherosclerosis. To test this hypothesis, we replaced 10% of the cholesterol of 7KC in an atherogenic diet (21%, saturated fat and 0.15% cholesterol) fed to male LDLr-/− mice for 12 weeks and measured hepatic apoA-1, PON-1 and SAA, plasma lipids and atherosclerosis. At the end of the study, plasma 7KC levels were in substantial amounts in the plasma of 7KC-fed mice, but not in the control group. However, there was no significant difference among the two groups in weight, plasma triglycerides and SAA, or lipoprotein distribution by FPLC. 7KC consumption was associated with lower plasma cholesterol levels, (0.08 ± 1 vs 570 ± 10 mg/dl; p < 0.02) and 35% lower hepatic PON-1 expression (0.04 vs. 0.04), with no difference in hepatic expression of apoA-1 or SAA. Atherosclerotic lesion area at the aortic sinuses was not different among the groups. We conclude that despite altering hepatic production of apoA-1, PON-1 and SAA in vitro, 7KC, when added to an atherogenic diet, had similar effects only on hepatic PON-1 expression, but did not increase atherosclerosis in male LDLr-/- mice. The lack of a difference in atherosclerotic lesion area may in part be explained by the adverse effect on PON-1 being offset by a reduction in plasma cholesterol. Higher doses of 7-ketocholesterol may be required to achieve the type of inflammatory changes observed in vitro.

Native C-Reactive Protein Does Not Increase Atherosclerosis Lesion Formation in Apolipoprotein E-Deficient Mice

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Background: Elevated C-reactive protein (CRP) levels are associated with endothelial activation and development of atherosclerosis. However, the conclusive proof of this association in vivo remains lacking. Some researchers reported a pro-atherosclerotic effect of CRP in ApoE-deficient mice (apoE−/−) while others did not. We assessed the hypothesis that continuous administration of azide- and endotoxin-free human native CRP (n-CRP) to apoE−/− mice would increase atherosclerotic lesion formation. Methods and Results: Twelve-week old male apoE−/− mice (n = 22) were used. Half of the animals received a continuous infusion of human n-CRP (20.4 μg/mice/day) for four weeks using osmotic pumps; the other half received vehicle only for 4 weeks. Mice were fed Purina 5001 rodent diet ad libitum. Then atherosclerosis was evaluated in aortic roots after Oil Red-O staining, and in complete aortas after Sudan IV staining. The extent of atherosclerotic lesion in the aortic root was not significantly different between the groups (CRP: 29.60 ± vs. Control: 29.4 %, p = 0.17). However, when measured throughout the whole aorta, the size of the lesion in mice treated with CRP was significantly smaller compared to control animals (CRP: 7 % vs. Control: 9.2%, p = 0.0029). Serum levels of soluble ICAM-1, VCAM-1, E-selectin and P-selectin were not significantly different between groups (p > 0.117). Conclusions: We demonstrated that continuous infusion of human n-CRP to apoE−/− mice did not increase atherosclerotic lesion formation, but, surprisingly, was associated with reduction of lesion formation throughout the whole aorta but not in the aortic root.

Local Production of Lp-PLA2 and Lysophosphatidylcholine in the Coronary Circulation: Association with Early Coronary Atherosclerosis and Endothelial Dysfunction in Humans

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Background: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a novel biomarker and participant in vascular inflammation. Inflammation is also associated with coronary atherosclerosis. We tested the hypothesis that local coronary production of Lp-PLA2 is enhanced in patients with early coronary atherosclerosis and associated with local endothelial function. Methods: Coronary angiography, blood flow, flow reserve, endothelial function, and intravascular ultrasound analysis were obtained in 30 patients with mild coronary artery disease. Plasma samples were collected simultaneously from the left main coronary artery and the coronary sinus for measurement of Lp-PLA2, its active byproduct and mediator lysophosphatidylcholine (lysPC), and C-reactive protein (CRP). Results: Fifteen patients had mild coronary atherosclerosis and 15 had no evidence of atherosclerosis by ultrasound (controls). Hemodynamic parameters and cholesterol profile were similar in both groups. Arterial Lp-PLA2 levels were similar in early atherosclerosis and controls (246 ± 19 vs. 230 ± 19 ng/ml). Contrarily, Lp-PLA2 net production across the coronary circulation was higher in patients with early atherosclerosis compared to controls (P = 0.04); the Lp-PLA2 product (P = 0.001) and correlated with the percent atheroma volume (r = 0.37, p = 0.04). LysPC net
production was also higher in patients compared to controls (74 ± 273 vs. -786 ± 273, p = 0.03) and correlated with coronary endothelial dysfunction (r = 0.5, p = 0.005). CRP net production was not significantly different between the groups. Conclusion: Early coronary atherosclerosis in humans is characterized by local production of Lp-PLA2. Local coronary generation of lysoPC, a product of Lp-PLA2, is then associated with coronary endothelial dysfunction. These results support the role for Lp-PLA2 in the mechanism of regional vascular inflammation and early atherosclerosis in humans.

P513 The Effect of Sphingomyelin Biosynthesis Inhibition on Lipid Absorption in Mice
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Serine palmitoyltransferase (SPT) is the rate-limiting enzyme in the sphingomyelin (SM) biosynthesis pathway. Mammalian SPT is composed of 2 different subunits, Sptlc1 and Sptlc2. Our previous study showed that mice orally treated with myriocin, a specific SPT inhibitor, reduces SM, cholesterol, and triglyceride levels in the circulation, while intraperitoneal treatment reduces only SM levels. Since oral administration of myriocin has a direct effect on the gastrointestinal tract, as suggested by a previous report, we hypothesized that the decrease of cholesterol and TG levels in the circulation might be due to a reduction in lipid absorption. We carried out cholesterol absorption studies on wild-type and apoE knockout (KO) mice, with or without oral myriocin administration, using the conventional fecal dual-isotope ratio method. The approach involved the gavage of a single bolus of 0.1 uCi [14C] cholesterol and 0.2 uCi [3H]sitostanol, together with 1 mg cold cholesterol in 30 uI olive oil. We observed that, after myriocin treatment, wild-type or apoE KO mice absorbed significantly less radiolabeled cholesterol than their controls (40% and 61%, respectively). The amount of radiolaabeled cholesterol assimilated into the circulation was significantly reduced in these mice, compared to their controls (63% and 82%, respectively). Moreover, myriocin treated mice absorbed significantly less radiolaabeled triglyceride than their controls (36% and 46%, respectively). More importantly, we found that heterozygous Sptlc2 KO mice, with less than 50% SPT activity in the small intestine, absorbed significantly less cholesterol (44%, p < 0.01) and contained significantly less radiolaabeled cholesterol and [3H]glycerolipids in the circulation (46% and 51%, respectively) than control mice. These results indicate that SPT is involved in intestinal cholesterol and triglyceride absorption. Manipulation of SPT activity by either specific inhibitors or gene therapy might provide a novel alternative treatment for dyslipidemia.

P514 Spontaneous Atherosclerosis in Old LPL-Deficient Mice with Severe Hypertriglyceridemia on a Normal Chow Diet
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Although type III hyperlipidemia is characterized by high levels of chylomicrons, we recently identified a mouse model of old age atherosclerosis in LPL-deficient (LPL-/-) mice. In these old mice, lipoprotein uptake and foam cell formation are increased in the vascular wall, suggesting that LPL-/- mice may be useful in dissecting the pathogenesis of atherosclerosis. We have previously shown that glucocorticoids specifically destabilize MCP-1 mRNA in aortic explants and in cultured VSMC. Similarly, addition of GR blocked the degradation of MCP-1 mRNA in aortic extracts in a concentration-dependent fashion. Conclusion: These results show that Dex treatment induces an MCP-1 mRNA degradation activity in rat aorta in vivo. Further elucidation of this mechanism may provide novel approaches to regulate vascular MCP-1 expression.

P515 Dexamethasone Induces MCP-1 mRNA Destabilizing Activity in Vivo in Rat Aortas
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Introduction: Monocyte chemotactic protein-1 (MCP-1) plays an important role in the pathogenesis of atherosclerosis. We have previously shown that glucocorticoids specifically destabilize MCP-1 mRNA in cultured vascular smooth muscle cells (SMC) by a glucocorticoid receptor (GR)-dependent mechanism. The purpose of this study was to determine whether this phenomenon also occurs in vivo. Methods: Sprague-Dawley rats were treated with Dexamethasone (Dex) 1mg/kg i.p. 24hrs and 1hr before sacrifice. Aortic media were isolated and S-100 cytoplasmic extracts were obtained as done for cell culture. Extracts were incubated with pre-titrated radiolabeled MCP-1 mRNA and analyzed by 4% polyacrylamide gels. Protein content was determined by Bio-Rad assay. Results: Extracts from Dex-treated rats induced a more rapid degradation of radiolabeled MCP-1 mRNA (t½ = 10min) as compared to extracts containing equal amount of proteins from untreated rats (t½ > 60min). Experiments were done to determine whether the Dex-sensitive activity in rat aorta was identical to that described in cell culture. We previously demonstrated that the initial 224 nucleotides of the MCP-1 mRNA contain the Dex-sensitive region. Similarly, extracts from Dex-treated rats degraded the 1-224 region, but not other Dex-insensitive region. Like in cell culture, the Dex sensitive degradation activity was heat unstable and sensitive to proteinase K. We have previously shown that exogenous GR blocked the degradative activity of Dex-treated cultured VSMC. Similarly, addition of GR blocked the degradation of MCP-1 mRNA in aortic extracts in a concentration-dependent fashion. Conclusion: These results show that Dex treatment induces an MCP-1 mRNA degradation activity in rat aorta in vivo. Further elucidation of this mechanism may provide novel approaches to regulate vascular MCP-1 expression.
developing rat brain, was found to be upregulated by approximately 10 fold in the aorta of dd/mm by 2 fold in the aorta of high fat fed mice. The neuritin protein was selectively increased in the aortic endothelium of dd/mm mice, but expression in other tissues (pituitary, brain, heart, kidney) was not altered in diabetic mice. Infection of human aortic endothelial cells with a murin encoding adenovirus resulted in increased expression of a panel of NF-κB regulated inflammatory cytokines, chemokines, and adhesion molecule genes. Neuritin expression also augmented TNF-α induced IL-6 production in endothelial cells. Neuritinon expression activated ERK kinase in endothelial cells, which may be a mechanism by which neuritinon activates the NF-κB program of inflammation. In summary, we have discovered that neuritinon expression lead to an upregulation of specific inflammation genes in endothelial cells that have direct relevance to the pathogenesis of atherosclerosis. The upregulation of neuritinon in the vasculature of diabetic mice may be a mechanism by which diabetes accelerates athero-

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**Activated Factor XII Type A Is an Independent Predictor of All-cause Mortality in Patients Admitted With Chest Pain**

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**Background:** Activated Factor XII (XIIa) is a predictor of recurrent coronary ischemic events in patients following a myocardial infarction (MI). Recently, novel in-vivo types of XIIa have been assessed. We assessed the relation between admission levels of activated factor XII type A (XIIaA) and long-term all-cause mortality in a large, consecutive cohort of patients admitted with chest pain. **Methods:** Blood samples for XIIaA determination were obtained immediately following admission in 871 patients admitted with chest pain suspected of having a MI. Plasma XIIaA concentrations were determined by ELISA at admission. All cause mortality within each following admission in 871 patients admitted with chest pain.

**Results:** From 90 moderate to severe hypertensive patients and 90 age and sex matched healthy control subjects venous blood was taken to measure matrix Gla protein levels compared to age and sex matched healthy controls. Infection of human aortic endothelial cells increased in the aortic endothelium of db/db mice, but expression in other tissues (pituitary, developing rat brain, was found to be upregulated. **Conclusion:** XIIaA is a powerful and independent indicator of long-term all-cause mortality in patients admitted with chest pain and provides prognostic information above and beyond to that provided by conventional risk factors.

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**Lower Levels of Matrix Gla Protein in Patients with Moderate to Severe Hypertension in Comparison to Normotensive Subjects**

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**Background:** Medial vascular calcifications are common among patients with hypertension. The vitamin K-dependent protein, matrix Gla protein, is protective of vascular calcification. We hypothesized that in subjects at risk for calcification serum levels of the protective matrix Gla protein are lower. In this study we investigated circulating matrix Gla protein in moderate to severe hypertensive patients compared to normotensive control subjects. Methods and results: From 90 moderate to severe hypertensive patients and 90 age and sex matched healthy control subjects venous blood was taken to measure matrix Gla protein. Hypertensive patients had significantly lower circulating serum matrix Gla protein as compared to control subjects (5.5 nm versus 7.2 nM, P < 0.0001). Conclusions: In this study we demonstrate that moderate to severe hypertensives have lower circulating matrix Gla protein levels compared to age and sex matched healthy controls.

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**Arterial Macrophage Colony Stimulating Factor Influences Atherosclerotic Lesion Size by Regulating Monocyte Migration and Apoptosis**

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**Introduction:** Macrophage colony stimulating factor (M-CSF) regulates monocyte/macrophage survival, differentiation, proliferation and migration. M-CSF is highly induced by oxidized lipids in endothelial cells, smooth muscle cells and monocyte/macrophages. It was found that M-CSF deficient mice on an apo-e−/− or low-density lipoprotein receptor null (LDLR−/−) background show in excess of a 50-fold decrease in the size of atherosclerotic lesions. However, the mechanism by which the M-CSF deficiency contributes to lesion development is unclear. **Objective:** We examined the basis of the resistance to atherosclerosis of mice deficient in M-CSF and the contributions of macrophage-derived M-CSF on lesion formation. Methods: Since mice homozygous for a null allele of M-CSF do not breed on an inbred C57BL6/J background-sclerosis studies, we exhibited atherosclerosis in M-CSF heterozygous mice bred onto a hyperlipidemic LDL receptor null background. Atherogenic lipoprotein was examined for measures of apoptosis, proliferation and chemotaxis. The source of M-CSF influencing lesion development was examined using bone marrow transplantation. **Results:** Heterozygous mice on a C57BL6/J LDLR−/− background exhibited a ~50% decrease in lesion size (257,765 μm² vs. 29,655 μm²; n=7 vs. 153,812 μm²; n=9,511 Het; n=12). The mice had a 61% increase in the fraction of apoptotic (TUNEL positive) macrophages per unit of macrophage positive lesion area (0.310 ± 0.06 Wt; n=7 vs. 0.189 ± 0.023 Het; n=7). No differences in the frequencies of mitotically active cells (2.38 ± 0.76 Wt; n=4 vs. 4.22 ± 0.789 Het; n=6) were detected. In vitro studies indicated that neutralizing M-CSF decreased monocyte chemotaxis 37% (n=9 per group). Bone marrow transplantation studies showed that the source of the bone marrow, whether +/+, −/− or −/− for M-CSF, had no effect on the development of atherosclerosis, suggesting that endothelial cell-derived M-CSF is responsible for the effect on lesion development. Conclusions: Our studies indicate that likely mechanism for the effect of M-CSF on atherosclerosis include monocyte recruitment and macrophage survival and that sources of M-CSF other than monocyte/macrophages contribute to lesion formation.

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**Plasma Expression of Functional scFv Antibody Against OxLDL in C57BL/6 Mice Following Adenoviral Mediated Gene Delivery**

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Much evidence indicates that the oxidation of LDL plays an important role in the atherosclerotic process. The unregulated uptake of OxLDL by macrophage scavenger receptors is believed to accelerate atherosclerosis, and in vitro studies have shown that certain antibodies to OxLDL can inhibit the binding and uptake of OxLDL by macrophages. However, the in vivo consequences of directly expressing plasma high titer of intact antibody or antibody fragments capable of blocking the uptake of OxLDL have not been tested. Raised plasma titers of an antibody to OxLDL using gene delivery of its cDNA would allow us to directly determine the long-term effects of such a strategy on atherosclerosis. In this research, we created a human monoclonal antibody (517, which binds to MDA-LDL and Cu-OxLDL and blocks the macrophage uptake of OxLDL, into the single chain format (scFv) by molecular engineering. We demonstrated that it was secreted into the culture media and was biologically active when a eukaryotic expression vector harboring scFv-17 was transfected into HEK-293 cells. We further generated an adenovirus vector encoding the cloned scFv-17 gene and demonstrated that scFv-17 can be efficiently expressed and secreted into the plasma of C57BL/6 mice after intravenous gene transfer of 2.5 X 1011 virus particles. The plasma scFv-17 bound specifically to MDA-LDL and Cu-OxLDL but not to unmodified LDL and immunostained atherosclerotic lesions, similar to its parental Fab form. In summary, achieving plasma expression of an antibody using gene transfer technology is a novel way to study the effects of antibodies to OxLDL on atherosclerosis progression and could provide insights into mechanisms by which immunological mechanisms modulate atherogenesis. This strategy could eventually have therapeutic consequences.
Structural Transition from Pentamer to Monomer: A Mechanism That Finely Regulates the Reactivity and Behavior C-Reactive Protein Exerts in the Inflammatory Process of Atherosclerosis

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C-reactive protein (CRP) is an ancient protein with high phylogenetic conservation; no deficiency or amino acid sequence polymorphism of CRP has yet been identified in humans. Emerging evidence indicates that CRP has at least two conformationally distinct isomers, i.e. pentameric CRP (pCRP) and monomeric CRP (mCRP). Both CRP isoforms play roles in inflammation and presumably atherosclerosis[1–3]. However, the origin of mCRP in vivo and how it forms remains unclear. Here we show that acute phase protein, pCRP, could function like a fine modulator of sophisticated cellular or physiological systems remains elusive. The basic function of pCRP is largely dependent on surface or multivalent ligand binding, thus membranes enriched in phosphocholine ligands could be important sites for pCRP function. Our results(1) showed that upon immobilized binding, pCRP first converted to a metastable intermediate with partly retained native conformation, i.e. mCRP(p), which could subsequently detach from membrane with a slow dynamics leading to formation of mCRP. Such a stepwise structural transition is accompanied with obviously increased capacity to exert classical or modified CRP bioactivities. We hypothesize that pCRP isoforms represents a repertoire of functional mCRP, while mCRP isoforms represent functional state of CRP. The rapid pCRP→mCRP transition may be a reliable mechanism that amplifies the classical CRP functions and could contribute to “acute phase” response. The subsequent slow mCRP→mCRP transition represents the secondary phase of CRP function, and exert modified activities as shown by us and others (3,5,6). The abundance of available pCRP ligands, the transition dynamics, and the proteolytic sensitivity of mCRP, all restrict pCRP→mCRP transition to be primarily a locally process occurring in inflammatory loci and can serve as a buffering mechanism to allow this protein to be fine regulator without unwanted violent stimulation. 1. Pepys, MB, and Hirschfield, GM (2003) JCI111:1805 2. Verma, S, Devaraj, S, and Jalil, I (2006) Circulation 113:2135 3. Schwedler, SB, Filep, JG, Galle, J et al (Am J Kidney Dis 47:2124 3. Ji, S-R, Wu, Y, Zhu, L et al (2007) ATVB Poster Presentations

MMP-9 Deficiency Does Not Influence Atherosclerosis but Promotes Abdominal Aortic Aneurysms in Both Hypercholesterolemic and Angiotsin II-Infused Mice

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Objective: The role of matrix metalloproteinase-9 (MMP-9) in the development of atherosclerosis remains to be clarified. However, MMP-9 has been implicated in the development of experimental abdominal aortic aneurysms (AAAs). The purpose of this study was to define the role of MMP-9 deficiency on hypercholesterolemia- and Angiotsin-induced atherosclerosis and AAA formation in ApoE-/- mice. Methods and Results: ApoE-/- mice were developed that were either wild type or deficient in MMP-9, with all comparisons being performed between littermates. Mice were infused with either saline or AngII (1,000 ng/kg/min) via osmotic pumps for 8 weeks and fed a normal laboratory diet. MMP-9 deficiency had no effect on plasma cholesterol concentrations, lipoprotein-cholesterol distributions, or systolic blood pressure during saline or AngII infusion. MMP-9 deficiency had no effect on the size of atherosclerotic lesions during either saline or AngII infusion. Unexpectedly, AAAs were observed in the super-renal aorta (P < 0.0027) of saline-infused MMP-9 -/- mice. Furthermore, infusion of AngII led to significantly increased AAA formation (P < 0.006) and increased death due to rupture of the abdominal aorta (P < 0.004). To determine whether MMP-9 deficiency led to structural changes, the AAA prone region was studied for cellular content and matrix integrity. We also determined whether MMP-9 deficiency led to functional defects by performing contractility studies in aortic rings. No structural or functional changes were discernable in the AAA prone region that could account for the exacerbated disease. Conclusion: These studies provided the unexpected result that deficiency of MMP-9 lead to AAA formation in mice with hypercholes- terolemia alone, and increased the incidence and severity of AngII-induced AAAs. The lack of MMP-9 during the development of the mice may lead to circumstances that promote AAA formation.\n
Cilostazol and Atorvastatin Have Synergistic Effects on Endothelial Nitric Oxide Synthase Phosphorylation and Protection Against Ischemia-Reperfusion Injury

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Background: Cilostazol (ATV) limits myocardial infarct size (IS) by upregulating Akt and protein kinase A (PKA) which activate endothelial nitric oxide synthase (eNOS) via phosphorylation at Ser1177[1,2]. However, when given orally, high doses of ATV (10 mg/kg/d) are needed to achieve maximal protective effect. Cilostazol (CIL) is a phosphodiesterase III inhibitor, used for treating patients with peripheral arterial disease. Several studies have suggested that CIL stimulates NO production by activating PKA. Hypothesis: CIL and ATV may have synergistic effects on eNOS phosphorylation and IS limitation. Methods: Sprague-Dawley rats received 3-day oral: 1) water; 2) ATV 2mg/kg/d; 3) CIL 20mg/kg/d; 4) ATV + CIL. Rats underwent 30min coronary artery occlusion and 4h reperfusion (n=10 in each group), or hearts were explanted for immunoblotting without being subjected to ischemia. Area at risk (AR) was assessed by blue dye and IS by triphenyl-tetrazolium-chloride (TTC). Results: Body weight and the size of AR were comparable among groups. There were no significant differences among groups in mean expression of eNOS, tissue calcium levels were higher in the ATV+CIL group (45±6% vs 37±2.7% P<0.0001). Conclusion: The additive effect of CIL and ATV on eNOS expression and IS limitation, imply that the combination of CIL and ATV may have additive/plus therapeutic effect on preventing IS caused by acute ischemia-reperfusion injury.\n
Sirolimus Attenuates Angiotsin II Plus Diet-accelerated Atherogenesis and Abdominal Aortic Aneurysm Formation in ApoE-deficient Mice


Background: Sirolimus is a macrolide inhibitor of mTOR kinase that markedly attenuates transplant vasculopathy and neointimal hyperplasia in animal models and humans. The effects of sirolimus on atherosclerotic lesions and abdominal aortic aneurysm (AAA) formation require further characterization. Objective: We investigated the effects of sirolimus on atherosclerotic lesion development and AAA formation in apolipoprotein E deficient (apoE-/-) mice on a high lipid diet receiving an angiotensin II (angII)-infusion to accelerate vascular pathology. Methods: Male apoE-/- mice were placed on a proatherogenic diet for 4 wks and given a continuous infusion of angII (1 μg/kg/min) via osmotic minipump. Sirolimus (0.5, 1.0, 4.0 mg/kg, ip) or vehicle were administrated once daily for 4 wks. In a second study, apoE-/- mice received an angII infusion and proatherogenic diet for 8 wks. After 4 wks of infusion, sirolimus (1.0 mg/kg, ip) or vehicle were given once daily for the remaining 4 wks to assess the potential for lesion regression. Results: Abdominal aortic lesion area and histology, AAA characterization and plasma cytokine levels were assessed. Results: Daily ip injection of sirolimus significantly reduced atherosclerotic plaque area (en face analysis) in a dose-dependent manner at 4 wks (sirolimus: 3.1 ± 0.4 % at 1.0 mg/kg, vs vehicle: 12.9 ± 1.8 %, P < 0.0001) and attenuated progression of established lesions at 8 wks (sirolimus: 18.4 ± 2.3% vs vehicle: 37.6 ± 2.7 %, P < 0.0001). Conclusion:
markedly reduced the incidence (sirolimus: 0 %, vs vehicle: 32 %, P < 0.005) and severity of AAA as determined by aortic diameter (sirolimus: 0.74 ± 0.02 mm vs vehicle: 1.38 ± 0.25 mm, P < 0.005). Sirolimus prevented elastin disruption and reduced CD68 inflammatory cell infiltration in aneurysmal tissue. These effects were associated with an altered Th1/Th2 cytokine profile in vivo. Conclusion: Our data suggest that sirolimus reduces the development of atherosclerotic lesions and AAA in ang-infused apoE– mice and may prove to be beneficial in modulating the severity of inflammatory vascular diseases.

Quantitative Annual Effect of Atorvastatin on Size and Content of Noncalcified Plaques of Coronary Arteries Following Atorvastatin Treatment by Multislice CT

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Background: Intensive lipid-lowering treatment with Atorvastatin reduced progression of coronary atherosclerosis, confirmed by NUS. To quantify the annual effect of Atorvastatin on size and content of non calcified coronary plaques (NCP) using MSCT and comparing LDL cholesterol levels. Methods: 21 subjects (16 males, 35–79 years, median 69) with NCP by MSCT (Light Speed Ultra 16) were enrolled. All were asymptomatic to differentiate thrombus from NCP in coronary arteries. Following LDL measurements, all were given 10mg of Atorvastatin (2 were 5mg as LDL levels were already lower than 70mg/dl) for 1 year, and MSCT and LDL measurements were repeated. One remarkable NCP was selected in each subject and evaluated as representative of effect of Atorvastatin. The area and CT values of NCP, excluding calcified portions, were manually measured from axial or multiplanar reconstruction images under the same conditions. Results: 21 NCP (18 LAD, 2 LCx, and 1 RCA) were evaluated. The mean LDL level was 122 mg/dl at the first scan and significantly decreased to 96 mg/ml at the second scan (P = 0.05). The areas of NCP were 2–31 mm² (mean 11.8) at first-scan, and 2–32 mm² (mean 12.9) at second-scan. The mean areas of NCP were not significantly different between the both-scans (11.8 at first and 12.6mm² at second scan). The averages of CT values were 55H at first scan and 62H at second scan and the mean of SDs of CT values were 40H at first and 45H at second scan and both were significantly higher in the second scan (P < 0.05).

The CT decreased significantly positive correlation between ratios (%) of annual change in area to baseline area at first scan of NCP (y) and LDL cholesterol levels (x) after one year of Atorvastatin treatment (y = 0.0106x ± 0.2765, R² = 0.1514, P < 0.05). Conclusion: Using MSCT, we could quantify the effect of Atorvastatin to the size and content of NCP and compare those with LDL cholesterol levels. Atorvastatin may decrease area of NCP if LDL levels are sufficiently decreased. Also, it may increase CT values, which could suggest a change in NCP components. LDL levels may be an important factor in decreasing the area of NCP. Further studies are needed using 64-slice MSCT in a larger population with sufficient decreases in LDL levels.

Cyclic Bending Is a Major Contributor to Critical Stress Conditions Leading to Corona Plaque Rupture: 3D MRI-Based FSI Models and Mechanical Image Analysis

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Introduction: Mechanical forces play an important role in the complicated process of atherosclerotic plaque rupture. We have developed MRI-based 3D multi-component models with multi-sensor interactions (FSI) in order to perform stress/strain analysis for atherosclerotic plaques and to identify possible mechanical and morphologic indices for accurate plaque vulnerability assessment. Hypothesis: Cyclic bending of coronary arteries caused by heart motion may be a major contributor to critical stress conditions of atherosclerotic plaque leading to increased plaque rupture risk. Method: A 3D multi-component FSI model was introduced to evaluate the effects of cyclic bending on stress/strain distributions in coronary plaques using geometry re-constructed from a 3D ex vivo high resolution MRI data set (36 slices) acquired from a human coronary plaque. Blood flow was assumed to be laminar, Newtonian, and incompressible. Both vessel and plaque component materials were assumed to be hyper-elastic and isotropic. Cyclic arterial bending secondary to cardiac motion was introduced into the computational model by specifying a region of asymmetric repeat displacement. The displacement was adjusted to achieve desirable curvature changes. In vitro flow experiments using hydrogel stenotic tubes with cyclic bending were conducted to validate our models. Results: Computational simulations were conducted using the hydrogel stenosis model and the coronary plaque sample under a 70 –130 mmHg physiological pulsating pressure. Stress behaviors tracked at selected critical sites (thin cap and major stress sites) showed that cyclic bending caused 100–400% higher stress variations. Multi-component plaque structure and cyclic bending led to nonlinear compression/expansion and higher stress variations in the plaque. Conclusion: Our initial study indicates that cyclic bending affects stress variations in coronary plaques to the extent that it plays at least as important a role as blood pressure does and must be included in coronary models for accurate mechanical analysis and stress-based plaque vulnerability assessment. Additional studies using this new model are warranted.

Postischemic Neovascularization Is Modulated by the A1 Adenosine Receptor

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Arteriogenesis and angiogenesis play a critical role cardiovascular development and in adaptive responses to tissue ischemia associated with arterial stenosis. Peripheral arterial disease (PAD) affects over 7 million Americans and claims the limbs of over 100,000 of these annually. Adenosine is a purine nucleoside metabolite of ATP degradation released by all ischemic tissues, where it acts to limit tissue injury via multiple mechanisms, including angiogenesis. Many of the effects of adenosine are mediated through its interaction with four subtypes of G protein-coupled cell surface receptors: A1,A 2A,A 2B, and A3. Proangiogenic effects mediated by A1 adenosine receptors (AR) have been described, but the role of the A1 AR is unexplored. The aim of our study was to identify the role of the A1 AR in neovascularization of the ischemic hindlimb. We compared neovascular responses between wild type (WT) mice and mice with gene-targeted A1 AR-deficiency (A1 KO) following femoral transection. While this technique does not result in distal tissue necrosis in WT mice, 50% of the A1 KO animals developed digital gangrene on the ischemic side. Laser doppler perfusion imaging (LDPI) of the distal extremity on post-operative day 7 demonstrated a 40% reduction in perfusion index (ischemic/non-ischemic flow ratio) in the A1 KO (29 ± 4%, n = 7) versus WT mice (52 ± 8%, n = 6, p < 0.03). These observations were supported using endothelial staining in histologic sections of gastrocnemius muscle, in which we observed 55% fewer capillaries in A1 KO sections than in WT (p = 0.03). Delivery of A1 AR antagonist (WRG 5071) in WT mice following ischemia, resulted in a 30% attenuation in LDPI index (control 42 ± 11%, n = 4 vs. WRC 31 ± 2%, n = 8) and a 33% decrease in capillary number, suggesting that impaired neovascularization in A1 KO mice is the result of receptor deficiency and not artifact from manipulation of the targeted locus. Further support comes from contrast-enhanced ultrasound imaging in the proximal hindlimb day 3 following ischemia showing a 42% lower perfusion index in A1 KO (41 ± 4%, n = 4) versus WT (71 ± 4%, n = 4, p = 0.03) muscle. We conclude that the A1 AR plays a significant role in stimulation of ischemia-mediated neovascularization and is a potential therapeutic target for PAD.
Bone Marrow-Derived Stem Cells Engineered to Overexpress Prostaglandin Synthase Survive Under Hypoxia Condition and Enhanced Capillary Assembly Around Homing Site in Hind Limb Ischemia Model

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Mesenchymal stem cell (MSC) delivery contributes to collateral formation through cell incorporation into vessels and secretion of angiogenic cytokines like HGF, VEGF, and MCP-1 in a paracrine manner. Prostaglandin is a proangiogenic prostanoïd which has a multifactorial function such as antiapoptosis and antiangiogenesis in endothelial cells. To test whether cell therapy with MSC overexpressed PGI2 would enhance proangiogenic effect to lead to accelerated recovery from hindlimb ischemia to the delivery of MSC alone. Methods and Results: Mesenchymal stem cell (MSC) was homed by flushing femurs and transplanted with adenoviral vector encoding GFP alone or GFP and PGI2 (AdGFP and AdBiG: P: 150 M0 each). Cell cycle analysis assessed by FACS revealed that, under hypoxia condition (5%O2), apoptosis rate was reduced by 65% in P+GFP-transfected MSCs and promoted cell proliferation by 52% in accordance with the results of bcl-2 and bcl-xL protein overexpression confirmed by western blotting. C57BL/6J mice received gene or MSC injection to the adductor muscle 1 day after femoral artery ligation. Mice were divided into 4 groups (n=8 each) following injected content; vehicle, AdGFP (Ad-i+P, 4x109 PFU), MSC with AdGFP (MG), and MSC with AdBiG (M+P) (M: mesenchymal stem cell, P: prostaglandin). Histological treatment demonstrated increased blood flow recovery in mice of M+P groups (Ischemic/Non ischemic limb at 1, 2, 3 weeks; p<0.01 vs. control) and MG+P mice reached to peak blood flow by 7 days after surgery earlier than MG (p<0.05), while Ad-i+P group had no significant blood flow increase with edema in the legs. In mice of M+P, collateral formation at 2 weeks was accelerated than MG mice (p<0.05). (Table) Histological analysis of ischemic tissues revealed that capillary assembly was enhanced around homing sites with positive staining of HGF and VEGF. Conclusion: Local delivery of MSC overexpressed PGIS enhanced capillary assembly around homing sites via paracrine mechanism.

BENEFICIAL EFFECTS OF MSC+PGIS DELIVERY

<table>
<thead>
<tr>
<th>Blood Flow Recovery</th>
<th>Capillary Density</th>
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<tr>
<td>Control</td>
<td>Ad-GFP+PGIS</td>
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<tr>
<td>Ischemic/Non ischemic limb at 3 weeks</td>
<td>0.52±0.03</td>
</tr>
<tr>
<td>Capillary Density (/HPF, at 2 weeks)</td>
<td>99±8</td>
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Data: mean±SE, *p<0.05 vs control, #p<0.01 vs control, ¥p<0.05 vs MSC+GFP

Segetalin Inhibits Angiogenesis via a VEGF-dependent Process

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Introduction Angiogenesis is a physiological vascular process that can be implicated in tumour growth and metastasis. Growth factors such as Vascular Growth Factor (VEGF) are implicated in angiogenesis process. Segetalin is a cyclic peptide isolated from Vaccaria segetalis (carophyllaeaceae) seeds. Segetalin is a phytoestrogen used in Chinese pharmacopeia for amnorrhoea treatment. Recently, epidemiologic and experimental studies showed that phytoestrogen consumption reduced cardiovascular disease risk. Hypothesis : Segetalin molecules could inhibit angiogenic process and tumour growth by inhibiting VEGF secretion from breast cancer cells. Methods and results : Natural Segetalin (SA) and SA (1,2)-analogue were synthesized in our laboratory. SA was obtained from the natural compound by an alanine > tryptophan substitution. 1) Cell proliferation tests were performed using Human Umbilical Endothelial Cells (HUVEC), and breast cancer cells lines MCF7 and MDA-MB-231. No difference between SA or S1 treated cells and vehicle treated cells was observed. 2) At 10 μM and 100 μM, S1 only inhibits tube formation in a matrigel assay (an in vitro angiogenesis model). 3) Using cell culture supernatants, we observed that SA and S1 (10 μM and 100 μM) decreased by 60% VEGF production in MCF7 and MDA-MB-231 vs vehicle treated cells (p<0.008) (VEGF ELSA). Conclusion : SA and S1 do not modify HUVEC, MCF7 and MDA-MB-231 proliferation but inhibit tumour angiogenesis by decreasing VEGF production from MCF7 and MDA-MB-231. S1 does not inhibit endothelial cell differentiation.

Type 2 Diabetes Impairs Collateral Artery Enlargement More than Type 1 Diabetes in Response to Hind Limb Ischemia in Mice

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Objective: Diabetes is an important major risk factor for peripheral arterial disease. Whether Type 1 or Type 2 diabetes has similar effects on the vascular response to limb ischemia is unknown. We investigated the effects of type 1 versus type 2 diabetes on blood flow recovery, arteriogenesis, and angiogenesis after induction of hindlimb ischemia. Methods: Ischemia: Ischemia was induced in streptozotocin (STZ)-treated (Type 1 diabetes), db/db (Type 2 diabetes), and wild type C57BL/6J (control) animals. Laser Doppler flow index studies were performed in all three groups to determine blood flow recovery and the number of collateral arteries. Hematoxylin and eosin staining analysis was also performed to determine capillary density, collateral diameter and fat infiltration. Results: Blood flow recovery after hindlimb ischemia was less complete in both Type 1 and 2 diabetes than in wild type mice (p<0.05). Both types of diabetes showed significantly fewer collateral arteries and smaller diameters than did controls (p<0.05). Type 2 diabetic mice showed a greater impairment in blood flow recovery than Type 1 diabetic mice (p<0.05). This difference was not due to fewer numbers of collateral arteries or lower capillary density (p=NS), despite lower pre-ischemic capillary density in type 2 diabetic mice (p<0.05). Rather, Type 2 diabetic mice had collateral arteries of significantly smaller diameters than Type 1 diabetic mice (p<0.05). Despite very low perfusion in the ischemic limb, no gangrene was observed in type 2 diabetic mice. In contrast, muscle weight was significantly increased in type 2 diabetic mice due to extensive fat infiltration unlike in type 1 diabetic or control mice. Conclusions: Type 2 diabetic mice displayed a distinctly different response to hindlimb ischemia than Type 1 diabetic mice. Type 2 diabetic mice had a greater in-pairmannt in blood flow recovery than Type 1 diabetic mice because of less collateral artery enlargement. The extensive fatty infiltration of the ischemic muscle of type 2 diabetic mice has not been documented in any other disease model. The molecular mechanisms responsible for the differences in blood flow recovery and collateral artery enlargement between Type 1 & 2 diabetes remain the focus of ongoing studies.

In Vivo Imaging of Murine Vasodynamics by Fourier Domain Optical Coherence Tomography

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In vivo imaging of vessels can provide new insights in the regulation of vasodynamics. In contrast to established isometric force measurements, fourier domain optical coherence tomography (OCT) imaging allows the investigation of vasorelaxation and vasodilatation in the anatomical context of the vessel without preparation trauma. The murine saphenous artery is a suitable model for the in vivo examination particularly due to the small diameter, the extensive fatty infiltration of the ischemic muscle of the vessel lumen dynamics. The results of this feasibility study are summarized in the table (n=4, mean±SEM). In conclusion, fourier domain optical coherence tomography allows the imaging of the vasodynamics of murine vessels in vivo. Further studies using high-fat diet in atherosclerosis/sensitive mice models will extend our knowledge about diet-specific changes in vasodynamics.
Cryoimaging of Atherosclerotic Lesions for 3D Microscopic Identification

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Our objective is to obtain 3D microscopic characterization of large atherosclerotic vessel specimens in less time and expense than standard histological methods, the gold-standard for identifying tissue types. These data sets can be used to validate other imaging modalities such as MRI or for high throughput animal model studies. We used cryo-imaging developed at Case Western Reserve University where block face of frozen samples are imaged with bright-field and autofluorescence imaging. We hypothesized that the main tissues in atherosclerotic lesions, e.g., lipid, fibrosis, calcification, can be accurately identified using cryoimaging. A whole vessel segment up to 5 cm can be imaged and a 3D microscopic volume generated. To test this hypothesis we conducted a validation study. Common iliac arteries from 10 cadavers were removed at autopsy and frozen within 4 hours into a block of cryo-histological medium gel (IC7). Blocks were positioned on a large cryomicrotome stage and 2 mm rings were cut after imaging the block face with an episcopic microscope at 7.5x magnification (39 nM resolution) using bright-field and autofluorescence imaging. For each ring, standard histological preparations were done: either HA, elastic van Gieson, and Mallory trichrome, or oil-red-O. A total of 192 matched cryosections and histological sections were collected. From the comparison between cryomaging and histology, we established rules to identify tissue types. Color/autofluorescence levels were adventitia: red-textured/-++, media: pink/+ + + +, solid calcification: white-textured/- + + + +, lipid: yellow0, cholesterol cleft: yellow-brown/- + + + +, and connective tissue: pale-pink/- + +.

Figure 1: 3D multiplanar reformating. Corrected images were registered and a 3D Velmex isocube volume was generated. This volume could then be reformatted at arbitrary angles. Shown are axial (a), sagittal (b), and coronal (c) sections.

Figure 2: example of matched cryosection (top row, bright on the left and dark on the right) and matched histology (bottom row). AD: adventitia; MT: media; Ca: calcification; D: dense connective tissue.

Excess Plasma Cholesterol in Patients with Single Cardiovascular Risk Factor, Increases Select Phenotype Endothelial Microparticles Expression and Directly Correlates With Elevated Circulating Levels In Blood

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Background: In response to injury, endothelial cells (EC) were shown to release membrane-derived microparticles CD 31/+42b- and CD 62E derived microparticles CD 31/-42b- and CD 62E. With high (HC) plasma compared to normal cholesterol and negative control (each p<0.05). There was no effect on CD 62E+ induction (p=0.62). Correlation analysis between cholesterol, LDL levels and circulating EMP count revealed a consistently positive association only with CD 31/+42b and not with CD 62E+ phenotype.

Conclusion: Cholesterol rich plasma affects EC and induces select phenotype EMP release. HC, respectively LDL is independently and directly associated with increase to CD 31/+42b-EMP phenotype. Therefore, increased level, in correlation to thrombus and development count and EMP phenotype reflect the endothelial injury pattern associated with HC.

Nonhematopoietic NADPH Oxidase Regulates VCAM-1-dependent Leukocyte Migration in Vivo: A Role for Endothelial Cell NADPH Oxidase

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Leukocytes migrate from the blood into tissue by binding to and migrating across endothelial cells. One of the endothelial cell adhesion molecules that mediate leukocyte binding is vascular cellular adhesion molecule-1 (VCAM-1). VCAM-1 is expressed on endothelial cells at sites predisposed to atherosclerosis and the VCAM-1 knockout mouse is an embryonic lethal. In addition, the infiltration of eosinophils into the lung in experimental asthma is dependent on VCAM-1. We have reported that ligand binding to VCAM-1 activates endothelial cell NADPH oxidase for the generation of reactive oxygen species (ROS). These VCAM-1-stimulated ROS are required for VCAM-1-dependent leukocyte migration in vitro. Therefore, we examined whether endothelial-derived NADPH oxidase modulates VCAM-1-dependent leukocyte recruitment in vivo. To examine non-hematopoietic NADPH oxidase function in vivo, mice deficient in NADPH oxidase (CBBY mice) were irradiated and received wild type hematopoietic cells to generate chimeric CBBY mice. To examine VCAM-1-dependent leukocyte migration, the mice were challenged with the antigen ovalbumin (OVA) as this induces VCAM-1-dependent eosinophilia. In response to OVA, the chimeric CBBY mice had increased numbers of eosinophils bound to the endothelial luminal surface in the lung tissue and bronchoalveolar lavage. This occurred without changes in VCAM-1 expression or cytokine/chemokine levels (IL-5, IL-10, IL-13, IFN, or eotaxin) in the lavage fluids or lung tissue of OVA-challenged mice. There was no change in leukocyte infiltration into the lung that occurs independent of VCAM-1. Interestingly, the OVA-challenged chimeric CBBY mice had reduced airway hyperresponsiveness (AHR). The AHR in OVA-challenged chimeric CBBY mice was restored by bypassing the endothelium with intratracheal administration of eosinophils. These data suggest that VCAM-1 induction of NADPH oxidase in the endothelium is necessary for the VCAM-1-dependent leukocyte recruitment during inflammation. Moreover, these findings provide a basis for targeting VCAM-1-dependent signaling pathways in anti-inflammatory therapies.

Mst1 Plays an Important Role in TNFα-induced Apoptosis of Vascular Endothelial Cell

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Background: Apoptosis of endothelial cells (ECs) is observed in unstable atherosclerotic plaques and pseudoaneurysms to induce erosion of plaque that leads to formation of thrombus and development of acute coronary syndrome. Mst1, a member of mammalian sterile20-like family protein kinase, is cleaved and activated during apoptosis. Although staurosporine and genotoxic agents are reported to activate Mst1, no physiological stimuli reported to date activate Mst1 except for Fas/FasL signal. Tumor necrosis factor-α (TNFα) is one of the mediators inducing apoptosis, however, the role and activation of Mst1 in TNFα-induced EC apoptosis have never been studied.
examined. Aim: We examined whether TNF-α activates Mst1 in EC. Results: Western blot analysis for Mst1 showed that TNF-α induced cleavage of Mst1 in a time- and dose-dependent manner, which indicated the activation of Mst1 kinase activity. TNF-α-induced Mst1 activation was significantly attenuated by pretreatment with Z-DEVD-FMK, a caspase3 inhibitor, confirming the previous report showing that Mst1 is activated by caspase 3. However, downregulation of Mst1 by siRNA did not affect TNF-α-induced activation of caspase 3, which is not consistent with the previous data that Mst1 and caspase 3 constitute a positive feedback loop. Inhibitors for mitogen activated protein kinases (ERK, p38MAPK and JNK) did not affect TNF-α-induced Mst1 activation. Diphosphonodiamide, a NADPH oxidase inhibitor, and N-acetylcysteine, an antioxidant, abrogated the effect of TNF-α-induced activation of Mst1 and caspase3, suggesting a role of reactive oxygen species in the activation of Mst1. Nuclear staining with Hoechst33258 and fluorescence activated cell sorting (FACS) analysis showed that TNF-α and overexpression of Mst1 induced apoptosis of ECs. TNF-α-induced EC apoptosis was confirmed by introduction of siRNA for Mst1 but not for scramble RNA. Conclusion: These results suggest that TNF-α induces Mst1 activation through caspase 3 and oxidative stress, and that Mst1 plays an important role in the induction of TNF-α-induced apoptosis of ECs. Inhibition, induction of Mst1 in unstable plaque may reduce apoptosis of ECs and stabilize vulnerable plaque.

Flow Differentially Regulates the mTOR Pathway in Cocultured Endothelial and Smooth Muscle Cells

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Hemodynamic forces are powerful regulators of vascular endothelial cell (EC) and smooth muscle cell (SMC) biology and phenotype. Endovascular stents, in particular, are routinely used for atherosclerotic obstructive disease and yet the biology of local delivery has not been fully elucidated. We hypothesized that stent deployment alters local hemodynamics eliciting profound effects on the response of underlying SMC and recovery of damaged endothelium. Single cultures of EC or SMC, and sequentially layered SMC/EC co-cultures seeded on silicone tubes were exposed to coronary artery-like flow for 20 min or 24 h. Pertussis consisted of cytokine-containing media (VEGF, FGF-2, IGF-1 and EGF), in the presence and absence of the rapamycin analogue CCI-779 (10 nM). After flow exposure, cells were immediately harvested and fixed for analysis. mTOR signalling was evaluated by flow cytometric detection of phosphorylated S6 ribosomal protein (P-S6RP). Bare metal stents were expanded within tubes before exposure to flow. Endothelial recovery after stenting was assessed using microscopic examinations. Experiments were carried out in triplicate. P-S6RP expression in EC increased 2-fold by flow exposure, but not in SMC. Mean intensity fluorescence (MF) of isolated EC cultures under static conditions was 530±21, and after flow exposure increased to 1054±42 (p<0.001). Interestingly, no differences in mTOR activation were observed for SMC. In the sequentially layered SMC/EC co-cultures, the expression of P-S6RP markedly increased in COC+ EC under static (MF 980±14 p<0.001) vs single cultures of EC and flow (1578±66, p<0.002 vs single culture of EC). CCI-779, abrogated the effect of flow on mTOR signalling reducing P-S6RP to below basal levels both with (160±16, p<0.001 vs flow basal level), and without flow (151±6, p<0.001 vs static basal level). Stent endothelialization under flow in stents with inhibitor was 4.6-fold lower (4.8±0.9 cells/10^5 μm^2) than in stents without inhibitor (22.2±6.7 cells/10^5 μm^2, p<0.05). Our findings suggest that activation of the mTOR pathway on EC and SMC is differentially regulated by flow, extending our understanding of the integration of flow and autocrine/paracrine regulation of vascular repair.

In Vivo Human Lower-extremity Saphenous Vein Bypass Grafts Manifest Flow-mediated Vasodilation

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Objective: Physiological changes in lower extremity saphenous vein bypass grafts (SVG) used to measure SVG diameter before and 1 min after reactive hyperemia (RH) to determine the tryptic peptide QNLQS745PTSSR at m/z 1197.526 was detected in iNOS treated in vitro with NO. To test the hypothesis that B1R agonists induce activation of extracellular signal-regulated kinase (ERK) which then phosphorylates and activates iNOS, we cotransfected HEX cells with B1R and iNOS. B1R agonist des-Arg10-kallidin (DAKD; 100 nM) stimulated ERK activation, phosphorylation of iNOS on serine and generation of “super-high” output NO. ERK activation inhibitor PD98059 greatly diminished NO production and also inhibited phosphorylation of iNOS. Ser^296 in iNOS was identified as the critical residue phosphorylated by ERK using an in vitro kinase assay and MALDI-TOF mass spectrometry of tryptic peptides from iNOS immunoprecipitated from transfected HEK293 cells. A peak corresponding to the phosphorylated form of the tryptic peptide ONIOE^296FFISR at m/z 1197.526 was detected in iNOS treated in vitro with activated ERK or iNOS from cells stimulated with 100 mM DAKD, but not in iNOS from untreated cells or cells transfected with iNOS mutated at Ser^296. iNOS and ERK were colocalized in subcellular domains as determined by confocal imaging and also co-immunoprecipitated, indicating that they interact. Transfection of cells with S745A mutant iNOS eliminated phosphorylation and abolished the ability of B1R signaling to generate iNOS-dependent NO, but did not inhibit basal iNOS activity. In contrast, the S745D mutant (mimics phosphorylation) had much higher basal activity, but was not activated by DAKD. This is the first demonstration that iNOS can be acutely activated by receptor-dependent ERK signaling and reveals a previously unappreciated level of complexity in its regulation. This novel pathway may play an important role in inflammatory vascular disease.

Enhanced Injury Response of Murine Aortas from Mice Exposed to Cigarette Smoke

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Cigarette smoke exposure leads to the enhanced development AAA in humans and murine models. We hypothesized that aortas taken from mice exposed to tobacco smoke would have an enhanced cytokine response to matrix injury. Methods: Male C57 mice were subjected to smoke from 3 filterless research cigarettes a day for 6 days per week for 4 (N=5) or 6 weeks

Receptor-dependent Activation of Inducible Nitric Oxide Synthase in Endothelial and Transfected Cells: Critical Role of INOS Phosphorylation

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Endothelial-derived NO is an important mediator of vascular function. Under normal conditions, eNOS generates low levels of NO and is strictly regulated by a variety of mechanisms. Exposure of endothelial cells to inflammatory mediators induces expression of iNOS and the kinin B1 receptor (B1R). Once expressed, iNOS is thought to constitutively produce high output NO until it is degraded. Here we show that iNOS activity can be acutely upregulated by activation of B1R in human endothelial cells or transfected HEK293 cells to generate 2.5 to 3-fold higher NO. To test the hypothesis that B1R agonists induce activation of extracellular signal-regulated kinase (ERK) which then phosphorylates and activates iNOS, we cotransfected HEX cells with B1R and iNOS. B1R agonist des-Arg10-kallidin (DAKD; 100 mM) stimulated ERK activation, phosphorylation of iNOS on serine and generation of “super-high” output NO. ERK activation inhibitor PD98059 greatly diminished NO production and also inhibited phosphorylation of iNOS. Ser^296 in iNOS was identified as the critical residue phosphorylated by ERK using an in vitro kinase assay and MALDI-TOF mass spectrometry of tryptic peptides from iNOS immunoprecipitated from transfected HEK293 cells. A peak corresponding to the phosphorylated form of the tryptic peptide ONIOE^296FFISR at m/z 1197.526 was detected in iNOS treated in vitro with activated ERK or iNOS from cells stimulated with 100 mM DAKD, but not in iNOS from untreated cells or cells transfected with iNOS mutated at Ser^296. iNOS and ERK were colocalized in subcellular domains as determined by confocal imaging and also co-immunoprecipitated, indicating that they interact. Transfection of cells with S745A mutant iNOS eliminated phosphorylation and abolished the ability of B1R signaling to generate iNOS-dependent NO, but did not inhibit basal iNOS activity. In contrast, the S745D mutant (mimics phosphorylation) had much higher basal activity, but was not activated by DAKD. This is the first demonstration that iNOS can be acutely activated by receptor-dependent ERK signaling and reveals a previously unappreciated level of complexity in its regulation. This novel pathway may play an important role in inflammatory vascular disease.
(N=5). Littermate mice were not exposed to smoke for either 4 (N=5) or 6 weeks (N=6). At sacrifice, 2 mm rings were prepared from infrarenal aorta and placed in serum free culture media. After 4 hours, the media (CM) was removed, stored and replaced with media containing elastase (0.16 U/ml) for another 4 hours. All CM was assayed for 13 cytokines on a bead array apparatus. The ratio between the stimulated and unstimulated CM was calculated. Results: The production of cytokines from the unstimulated aortic explants was similar for all mice. Stimulation of aortas from control animals showed significant increases in the production of IFN-γ, IL-10, IL-12 and MCP-1. After 6 weeks of smoke exposure, elastase stimulation significantly increased production of the same cytokines as well as MIP-1α. By comparing the stimulated cytokine production between aortas taken from the smoke-exposed and control animals, we found all 5 cytokines were released in significantly higher concentrations by aortas from smoke exposed mice. The IL-1α response to stimulation was reduced by smoking, although the change was not significant. There appeared to be a dose-dependent trend between the duration of smoke exposure in vivo and cytokine response. Conclusion: Cytokine response of the aorta to matrix injury is significantly greater after in vivo tobacco smoke exposure.

Temperature and Blood Pressure Following Amlodipine Overdose: A Test of the Thermoregulatory-Vascular Remodeling Hypothesis

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BACKGROUND: The cardiovascular system participates in both blood pressure (BP) and temperature regulation. The thermoregulatory-vascular remodeling (TVR) hypothesis postulates that temperature homeostasis has precedence over BP homeostasis. As a result, salt ingestion creates conflict between BP and temperature homeostasis, for prompt vasodilatation to lower BP. Since vascular remodeling is irreversible, the baseline peripheral resistance increases incrementally following an ingestion of salt. After numerous episodes of salt ingestion, the baseline BP also rises. OBJECTIVE: A case of amiodipine overdose offered an opportunity to test one of the predictions of the TVR hypothesis: that vasodilators increase cutaneous blood flow, thereby accelerating heat loss and lowering the core body temperature. In order to avoid heat loss, salt ingestion elevates the BP until the kidneys excrete the excess salt. During these transient elevations in BP, peripheral resistance increases due to vascular remodeling. Since vascular remodeling is irreversible, the baseline peripheral resistance increases incrementally following an ingestion of salt. After numerous episodes of salt ingestion, the baseline BP also rises. OBJECTIVE: A case of amiodipine overdose offered an opportunity to test one of the predictions of the TVR hypothesis: that vasodilators increase cutaneous blood flow, thereby accelerating heat loss. Consequently, one would expect either a drop in body temperature and/or an increase in the metabolic rate. METHODS: Following the ingestion of 1000 mg of amiodipine, the temperature and BP of a single patient were monitored following presentation for emergency care, during the initial 24 hours of hospitalization, and during the final 24 hours of hospitalization. Temperature readings were available beginning 7 hours post ingestion, and then only intermittently, varying from once an hour to once every four hours. RESULTS: The BP dropped markedly between the 5th and 7th hours post ingestion, but then the BP rose steadily and normalized by 28 hours post ingestion. The temperature was normal at 7 hours post ingestion, declined gradually between the 23rd and 26th hours post ingestion, stabilized between the 26th and 31st hours post ingestion, then began to rise. CONCLUSIONS: During amiodipine overdose, a modest temperature decline lagged behind a marked BP decline. The BP normalized following medical therapy. As the BP rose, the temperature also rose, but lagged behind the BP increases. These results are consistent with the predictions of the TVR hypothesis.

A Novel Small Molecule Inhibitor Targeted to a Ubiquitin-Ligation Enzyme Slows Down Formation of Abdominal Aortic Aneurysms in a Rat Experimental Model

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The objective of the present study was to identify new therapeutics for abdominal aortic aneurysms (AAA). We used microarray expression profiling to identify biological pathways relevant to AAA pathology and to identify potential targets in these pathways against which to develop novel small molecule inhibitors. We identified the ubiquitin-proteasome system and targeted the ubiquitin ligation enzymes. Our approach is structural chemical-genetic ligand design to identify small molecule inhibitors of selected ubiquitin-ligation enzymes. The compounds were tested in the elastase-perfusion model for AAA in rats. Animals receiving only the vehicle (dimethylsulfoxide, DMSO; n=5) developed AAAs, while rats treated with doxycycline (n=19) and our novel inhibitor called 0464 (n=9) had smaller increases in aortic diameter 14 days following elastase-perfusion (comparison to DMSO group: p=0.05 for doxycycline group and p=0.05 for 0464 group). Histological analysis showed that elastin in the medial wall (Verheoef van Gieson stain) was degraded in DMSO aortas, whereas it remained intact in doxycycline and 0464-treated rats. The inflammatory cells were reduced in doxycycline and 0464 groups compared to controls. The detailed mechanistic target validation of the small molecule inhibitor 0464 is being undertaken. Together these results demonstrate that the novel small molecule inhibitor 0464 targeted against ubiquitin-ligation enzymes was effective in slowing down the growth of AAA in the rat elastase-perfusion model to the same extent as doxycycline.

Angiotensin II Infusion Results in Region-Specific Aortic Hypertrophy and Hyperplasia Requiring AT1a Receptor Activation of p47-phox and ID3 in a Pressure-independent Manner

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Objective: We previously demonstrated that angiotensin II (AngII) infusion leads to an AT1a receptor (AT1aR) dependent medial arterial hyperplasia as well as a medial hypertrophy in the thoracic and abdominal regions; independent of blood pressure. The objective of this study was to determine the mediators of this AT1aR-induced medial change. Furthermore, we assessed the mechanism responsible for AngII-mediated aortic hyperplasia. METHODS AND RESULTS: p47(phox) is a critical component of the NADPH oxidase complex, which is a key mediator of AngII-induced smooth muscle cell (SMC) hypertrophy. To determine its importance to the development of AngII-induced aortic medial thickening, male p47(phox)-/- mice (n=7/group) were infused with either saline, AngII (1,000 ng/kg/min), or noradrenalin (NE, 5.6 mg/kg/day) for 28 days (n=5 each). After the infusion, mice were perfusion-fixed and the ascending arch, thorax, supra, and infra-renal abdomen were sectioned serially. The medial thickness of aortic tissue sections (5–12 per region) was measured by image analysis. p47(phox)-/- mice were resistant to increased thickness during AngII-infusion, consistent with this subunit being a critical component of AT1aR signaling. Furthermore, p47(phox)-/- mice had greatly attenuated increases in systolic blood pressure (SBP) during AngII-infusion. NE infused mice had significantly increased SBP, but without medial expansion, suggesting the p47(phox)-/- effects are pressure independent. ID3 is a helix-loop-helix factor that induces AngII-mediated SMC proliferation. ID3-/- mice were infused with saline (n=6) and AngII (n=7) to ascertain if this mitogenic protein leads to the aortic arch hyperplasia. ID3 deficiency did not alter AngII-induced medial thickening, however, the AngII-induced hyperplastic response was attenuated in the ascending arch. Conclusion: The increase in aortic medial thickened, induced by AngII infusion, is mediated via AT1aR leading to activation of the p47(phox) subunit of NADPH oxidase, in a pressure independent manner. Furthermore, attenuation of the AngII-induced aortic hyperplasia in ID3-/- mice suggests that this protein is responsible for the mitogenic effect in this region.

Continuous Angiotensin II Infusion Promotes Progressive Expansion and Vascular Remodeling of Abdominal Aortic Aneurysms in Apolipoprotein E-/- Mice

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Objective: Chronic subcutaneous angiotensin II (AngII) infusion into mice leads to rapid initiation of abdominal aortic aneurysms (AAAs). The objective of this study was to determine whether prolonged AngII infusion led to AAT type progression and changes in pathogenesis. Methods and Results: Male ApoE-/- mice were infused with AngII (1,000 ng/kg/mim) by Alzet mini-osmotic pump for 28 days. Lumen diameters of the abdominal aortas were measured by high frequency (40 MHz) ultrasound. Mice with AAAs were assigned to 3 groups at 28 days. Group 1 was terminated (n=15); Group 2 was infused for a further 56 days with AngII (n=15); Group 3 was infused for a further 56 days with saline (n=13). An additional Group 4, without any infusion, was observed at 84 days. Infusion of AngII increased systolic blood pressure (SBP) by ~30 mmHg throughout the infusion period in Gp 2. However, the removal of AngII led to an immediate return to baseline SBP in Gp 3. Groups 1–3 exhibited increased aortic lumen dimensions at 28 days of infusion. Lumen dimensions were further increased during continued AngII infusion in Gp 2, but remained at 28 days values in saline-infused Gp 3 mice. At 28 days of AngII infusion, the expanded lumen was frequently associated with adventitial thickening, thrombus and macrophage infiltration (CD68+ cells). At 64 days of AngII infusion, profoundly dilated aortas were associated with extensive wall thinning and neomedial areas composed of CD68+ cells. Thrombus was not evident in AAAs from mice infused with 64 days of AngII. Gp 3 mice infused with saline also had thinned aortic walls with reduced thrombus; however, CD68+ cells were less prevalent. No abnormal pathology was noted in abdominal aortas of mice infused with saline for 84 days. Conclusion: Continuous AngII-infusion led to progressive expansion of the aortic lumen. These dilated AAAs exhibited different features, including thinned walls, and profound macrophage infiltration than normal vessels.
Nox1 Knockout Does Not Affect Angiotensin II-dependent Chronic Hypertension and Cardiac Hypertrophy

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Background: The gp91phox (Nox2)-containing NAD(P)H oxidase is the major source of reactive oxygen species (ROS) in the cardiovascular system. Inactivation of gp91phox blunts hypertension and cardiac hypertrophy in acute studies of Ang II-infused animals, but not in mice in which the endogenous renin-angiotensin system (RAS) is chronically upregulated. The role of other Nox isoforms, such as Nox1, in chronic Ang II-dependent hypertension is unknown.

Objective: In the present study we sought to determine whether Nox1 plays a role in the activation of redox-sensitive pathways leading to development of hypertension and cardiac hypertrophy in a model in which the RAS is chronically upregulated.

Methods and results: Nox1-deficient mice and transgenic mice expressing high levels of active human NAD(P)H oxidase were crossed and four genotypes generated: control, TTHR(Ren) transgenics (TTHR(Ren)), Nox1-deficient (Nox1), and TTHR(Ren) transgenic Nox1-deficient (TTHR(Ren)/Nox1). Blood pressure, cardiac mass, and cardiac fibrosis were increased in TTHR(Ren) versus controls. This was associated with increased blood pressure and cardiac hypertrophy in TTHR(Ren)/Nox1 mice. Nox1 deficiency in TTHR(Ren)/Nox1 mice decreased target organ damage and blood pressure. We have previously reported that Nox1-containing NAD(P)H oxidase may not be important in hypertension and cardiac hypertrophy in a model in which the endogenous renin-angiotensin system is chronically upregulated.

Inhibition of Glycogen Synthase Kinase-3β Decreases Vascular Smooth Muscle Cell Growth in Vitro Through Modulation of Notch Signaling

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Vascular cell fate decisions are hallmarks of the vascular response to injury and play a crucial role in the pathogenesis of vascular disease. Glycogen synthase kinase-3β (GSK-3β) has been implicated in vascular morphogenesis and remodelling of the embryonic vasculature. We have previously reported that Notch plays a major role in the regulation of adult vascular cell smooth muscle cell (VSMC) fate decisions.

In the present study, we hypothesized that the growth-inhibitory effects of PPARα/δ agonists may involve an inhibition of telomerase activity, which controls key cellular functions including replicative senescence, cell proliferative senescence and cell death. We have previously reported that PPARα/δ agonists inhibit telomerase activity in VSMC through a p16 INK4a-dependent mechanism, which provide a previously unrecognized mechanism for the anti-proliferative effects of PPARα/δ ligands in VSMC. They support the concept that the PPARα/δ agonist pathway may constitute a novel therapeutic target for the inhibition of VSMC proliferation in the prevention of cardiovascular disease.
and pDES implantation. Gene expression profiles were generated from five male donor LIMAs divided into three parts: non-stent control, BMS, or paclitaxel DES. Less than 10% of the probe sets were differentially expressed in the BMS and pDES groups, compared to control, and in pDES compared to the BMS. No more than 3% of all genes were differentially regulated among the three groups. Genes involved in cell growth were up-regulated in both BMS and pDES. However, paclitaxel DES displayed a pronounced effect on the cell cycle re-endothelialization and maturation within the vascular wall. These data can help guide future work to optimize DES, by developing parameters which further promote endothelial healing.

ALDO Aldosterone Increases Oxidant Stress to Decrease INOS-stimulated Soluble Guanylyl Cyclease Activity

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Elevated levels of aldosterone (ALDO) are associated with impaired vascular reactivity. In vascular endothelial cells, ALDO modulates this effect by increasing reactive oxygen species (ROS) and decreasing bioavailable nitric oxide (NO). However, the effect of ALDO on vascular responses in vascular smooth muscle cells (SMC) remains unknown. We hypothesized that ALD impairs SMC relaxation by increasing ROS to decrease soluble guanylyl cyclase (sGC) activity. To test the effect of ALDO on ROS, bovine aortic SMC were treated with ALDO (10-9–10-7 mol/L) or a vehicle (V) control for 24 h and ROS was measured by 6-carboxy-2'- indo-1-36 fluorophore. Compared with V-SCM, ALDO-SCM showed a concentration dependent increase in ROS (117.9± 5.7: vs 124.5± 6.1 vs 144.9± 4.6: p<0.01); treatment with apocynin (30 μM/L), a specific NADPH oxidase inhibitor, decreased ALDO-induced ROS formation (95.7± 5.0% vs 98.4± 5.0%: p=0.05). The effect of ALDO on endogenous NO-sGC signaling in SMC, INOS was induced by cytokines (C-SMC) for 24 h. INOS protein expression was increased in C-SCM compared to V-SCM (0.26± 0.02: 0.019 vs. 0.020± 0.005: pmol/μg protein: p<0.01). Exposure to ALDO did not increase INOS protein expression or nitrite formation further in C-SMC; however, ALDO decreased INOS gene expression by 50.7% (0.014± 0.0019 vs. 0.002± 0.002 acetylated cGMP pmol/μg protein: p=0.05). Apocynin restored INOS stimulated cGMP production in ALDO-treated C-SCM (0.007± 0.002 vs. 0.014± 0.003 pmol/μg protein: p<0.01) without influencing nitrite levels. These studies demonstrate that ALDO promotes ROS generation by NADPH oxidase in SMC, when INOS is induced, this effect is associated with decreased sGC activity and cGMP levels without influencing NO byproduct formation. These findings suggest that ALDO-induced ROS leads to an uncoupling of INOS-mediated NO bioactivity and, thereby, may impair SMC relaxation.

Heme Oxygenase-1 Prevents Aldosterone-elicted Vascular Senescence Through Mechanisms Involving SIRT1/p53/p21 Pathway

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In yeast, restriction of calorie extends life span by increasing activity of Sir2, an NAD+-dependent deacetylase. SIRT1, a human homolog of Sir2, has been reported to inhibit p53, which is one of the key players in stimulating cellular senescence in vascular wall. Heme oxygenase is a microsomal enzyme that catalyzes the degradation of heme into biliverdin, which is subsequently reduced to bilirubin, free iron and carbon monoxide (CO). Induction of heme oxygenase-1 (H0-1) has been potentially associated with cellular protection, especially against oxidative insults, which promote age-related changes. In this study, using smooth muscle cell-directed H0-1 over-expression mouse (H0-l Tg), we investigated anti-aging effect of H0-1 on cardiovascular system in aldosterone/high salt (ALD-treated) mice. Saturation of ALD promotes in vivo cellular senescence as determined by senescence-associated beta-gal staining as well as increased expression of p53 and p21 in the aorta of wild type mice (WT). Consistent with these findings, expression of SIRT1 was markedly reduced, while inflammatory cytokines were induced and eNOS was decreased, respectively, in the aorta of ALD-treated WT. In contrast, there are no significant changes in these senescence-related markers, and expression of SIRT1 was not reduced in ALD-treated H0-1 Tg. Taken together, these findings indicate that vascular H0-1 counteracts vascular senescence through restoration of SIRT1 as well as inhibition of p53/p21 pathway in aorta. Vascular H0-1 can be a new therapeutic target against age-related cardiovascular diseases.

Enos Gene Expression by Adenosine Prevents Angiotensin II-induced Vascular Smooth Muscle Cell Hypertrophy Through Selective Inhibition of the Rho/Rho-Kinase Pathway

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Enhanced angiotensin II (AngII) actions are frequently associated with endothelial dysfunction, which is characterized as decreased nitric oxide (NO) availability. Although endothelial NO synthase (eNOS) is believed to antagonize vascular remodeling induced by the AT1 receptor, the exact signaling mechanism remains controversial. Therefore, we investigated a possible signal cross talk between eNOS and AT1 along with their impacts on vascular hypertrophy by using vascular smooth muscle cells (VSMCs) infected with adenovirus encoding the eNOS gene. In VSMCs infected with eNOS adenovirus, basal G kinase activity was enhanced as detected by enhanced VASP Ser239 phosphorylation. AngII-induced VSMC hypertrophy as judged by protein synthesis as well as by cell volume was again markedly inhibited by eNOS adenovirus. These effects are accompanied with selective inhibition of the Rho/Rho-kinase (ROCK) cascade. The downstream effect components of ROCK1, p190 Rho-kinase and a novel light chain phosphorylation induced by AngII, were inhibited by the eNOS expression. From these data, we conclude that under eNOS gene transfer, NO/cGMP production and the resultant G kinase activation appear to prevent AngII-induced VSMC hypertrophy by selective inhibition of the ROCK/ROCK pathway. These data suggest a novel molecular mechanism of endothelial dysfunction leading to vascular remodeling through selective induction of the Rho/ROCK cascade.

The Reversible Oral P2Y12 Antagonist AZD6140 Inhibits ADP-induced Contractions in Mouse Aorta in Addition to Established Inhibitory Effects on Platelet Aggregation

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Introduction. Platelet P2Y12 receptor has long been used as a target for antithrombotic drugs of the thiophenopyridine family such as clopidogrel and ticlopidine, which are prodrugs whose bioactive metabolites bind irreversibly to P2Y12 receptor. AZD6140, a reversible oral P2Y12 antagonist with no antagonism for metabolic activation, is being studied for its potential to prevent thrombotic events in patients with acute coronary syndromes (ACS). Clinically, AZD6140 showed a greater inhibition ofurine clopidogrel, with a similar incidence of total bleeding events. P2Y12 receptors have also been shown on vascular smooth muscle cells (VSMC), where they mediate a contractile function after stimulation by ADP; this could contribute to local vasospasm and poorer outcomes in ACS. Objective. To elucidate if AZD6140, in contrast to clopidogrel, can act on VSMC and thereby inhibit ADP-mediated contractions. Methods. Nine female mice were used: 5 were pretreated with clopidogrel 50 mg/kg the day before, and 2 hours before the experiment; 4 were not pretreated. Thoracic aorta sections obtained from all mice were dissected from connective tissue and denuded. Ring segments (7 or 8 per mouse) were mounted into temperature-controlled tissue baths with physiological Krebs buffer. AZD6140 10 μM or DMSO 1:1000 as control was added; after 20 minutes, segments were precontracted with 10 nM norepinephrine, followed by the P2Y12 agonist 2-MeSADP (10 μM). Results. Mean 2-MeSADP-induced contraction (% maximal contraction induced by 60 mM KCl) was reduced to 41.1% (P<0.002) and 32.3% (P<0.001) with AZD6140. Mean 2-MeSADP-induced contraction (% maximum contraction induced by 60 mM K+) in clopidogrel-treated and untreated DMSO control groups was 64% and 59%, respectively; this was reduced to 32% (P<0.002) and 33% (P<0.015), respectively, with AZD6140. Conclusion. AZD6140 blocked ADP-induced vasoconstriction mediated by P2Y12 receptors in denuded mouse aortic rings, regardless of in vivo pretreatment with clopidogrel. This effect of AZD6140 could potentially modulate vasoactivity mediated by locally concentrated ADP of vasoconstricition. In vivo. Further investigation is required to examine this question.

TGF-b Increases Intimal Hypertrophy After Vascular Injury Through Smad3-Mediated Stimulation of Cell Proliferation

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Introduction: Intimal hyperplasia is associated with the transformation of vascular smooth muscle cells (VSMC) from a quiescent to a proliferative phenotype. TGF-beta has been shown to be a critical factor in the development of intimal hyperplasia. We hypothesize that TGF-beta, through Smad3 signaling, increases intimal hyperplasia by stimulating VSMC proliferation. Methods: Male Spague-Dawley rats underwent left carotid balloon injury followed by intraluminal delivery with adenovirus expressing Smad3 (AdSmad3) or control (LacZ or empty virus). Injured arteries were harvested at specified time points and subject to histological analysis. Results: Endogenous Smad3 is upregulated in the rat carotid artery after balloon injury. Further upregulation of Smad3 by adenovirus-mediated gene transfer increased intimal hyperplasia. The role inhibition of Smad3 signaling via overexpression of Smad7 decreased neointimal formation. Upon immunohistochemical analysis, PCNA staining was increased in Smad3 infected arteries and decreased in Smad3 infected arteries, thus suggesting a role for Smad3 signaling in cell proliferation. The expression of Smad3 by adenovirus-mediated gene transfer increased intimal hyperplasia. Our data provide a strong role for Smad3 in regulating cell proliferation and further upregulation of Smad3 led to increased expression of P2Y12 receptor on the endothelial cells. We found by western blotting that upregulation of Smad3 results in serine-10 phosphorylation of p27 and a subsequent decrease in total p27. Conclusions: Upregulation of the TGF-beta signaling protein Smad3 following arterial injury causes quiescent VSMC to re-enter the cell cycle by phosphorylation and subsequent degradation of the cyclin dependent kinase inhibitor p27.
Abundance and Plasticity of Circulating Stem Cells in Patients with Abdominal Aortic Aneurysm

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Background: Abdominal aortic aneurysms (AAAs) are characterised by progressive degenerative changes in the wall of the aorta, leading to a structurally weaker vessel which is at risk of potentially fatal rupture. Recently, we reported that aneurysmal rupture correlated with medial neo-vascularisation. As neo-vascularisation is most likely to require engraftment and differentiation of a circulating stem cell population, we have investigated the role of stem cells in the pathophysiology of AAA. Objectives: We aimed to evaluate the number of circulating stem cells, and then investigate the effects of EPC in vitro conditions on adhesion, migration and proliferation. Methods: Venous blood samples are collected from patients undergoing endovascular repair of AAA, CD133+/ Sca-1 cells are isolated from peripheral blood mononuclear cells (PBMCs) using magnetic cell sorting of CD133 antibody labelled cells (Miltenyi Biotech). CD133+/Sca-1 cells are cultured in HUVEC MEM, 2 day and 5 day PBMC conditioned medium. Both 2 day and 5 day PBMC conditioned medium preferentially support the growth of CD133+/ Sca-1 cells. Conclusion: The number of circulating CD133+/ Sca-1 cells are increased in patients with AAA compared to normals (2.43 versus 1.25%, p = 0.008). Plasma VEGF concentration is increased compared to normals (33.2 ± 0.36 vs 18.6 ± 0.14, p < 0.002). GCSF demonstrated the same trend (9.7 ± 0.02 vs 6.8 ± 0.02, p = 0.03). CD133+/ Sca-1 cells were cultured in HUVEC MEM, 2 day and 5 day PBMC conditioned medium. Both 2 day and 5 day PBMC conditioned medium preferentially support the growth of CD133+/ enriched cells in comparison to standard HUVEC-MEM. Conclusions: The number of circulating CD133+/Sca-1 cells increases in patients with AAA. We have found that PBMC-conditioned medium optimises the growth conditions of CD133+/ enriched cells. Mediator substances such as growth factors and cytokines secreted by the PBMCs promotes cultivation of these rare progenitor cells in preference to endothelial cell growth factor alone.
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