Abstract—Atherosclerosis is a major vascular complication of diabetes and the primary cause of mortality in persons with this disease. Metabolic abnormalities related to the Insulin Resistance Syndrome or Metabolic Syndrome may importantly contribute to the increased risk of atherosclerosis associated with diabetes. Thiazolidinediones (TZDs) are oral insulin sensitizers in broad clinical use that enhance insulin-stimulated glucose uptake into skeletal muscle. TZDs can also improve cardiovascular risk factors and exert direct effects on vascular cells to potentially retard the atherosclerotic process. Direct vascular effects of TZDs likely result from their activity as ligands for the nuclear receptor, PPARγ. All of the major cell types in the vasculature express PPARγ, including intimal macrophages and vascular smooth muscle cells (VSMCs) in human atheroma. TZDs block VSMC growth by inducing cell cycle arrest in G1 through an inhibition of retinoblastoma protein phosphorylation. Migration of monocytes and VSMCs is also inhibited by TZDs, possibly through decreased matrix metalloproteinase production. Activation of PPARγ by TZDs in macrophages induces ABCA1 transporter expression to promote reverse cholesterol transport. These antiatherogenic activities may also occur in vivo because TZDs have been shown to inhibit lesion formation in several animal models. Thus, TZD activation of PPARγ may protect against atherosclerosis both by normalizing proatherogenic metabolic abnormalities of the insulin resistance/diabetes milieu and through an inhibition of vascular cell growth and movement. (Arterioscler Thromb Vasc Biol. 2001;21:1891-1895.)

Key Words: atherosclerosis ■ PPARγ ■ cell cycle ■ thiazolidinedione

Atherosclerosis is increasingly recognized as a major, if not the major, complication of diabetes. It is the number one cause of mortality in people who have diabetes.1–3 Hyperglycemia contributes, in part, to accelerated vascular injury in diabetes, but prediabetic patients with impaired glucose tolerance also have accelerated rates of atherosclerosis.4–6 Resistance to insulin’s action to stimulate glucose uptake into skeletal muscle is not only associated with abnormalities in glucose tolerance, but with a well known dyslipidemia (low HDLC and high triglycerides) and hypertension, which are themselves defined as risk factors for atherosclerosis.7,8 In addition, insulin resistance seems to be a state of increased oxidation, thrombosis, and vascular inflammation with elevated, circulating levels of small, dense low-density lipoprotein cholesterol (sd LDLC), plasminogen activator inhibitor 1 (PAI-1), and increased plasma levels of C-reactive protein (CRP).9–12 This collection of abnormalities is called the Insulin Resistance Syndrome or Metabolic Syndrome.7,8

Insulin resistance also represents a state of imbalance between two key cell signaling pathways that mediate insulin’s actions: 1) the phosphatidylinositol-3 kinase (P13K) pathway that mediates insulin’s function as a growth factor.16,17 More recently, the ERK MAPK pathway was shown to play a key role not only in the growth of vascular cells, but also in the migration of the vascular cells and in vascular PAI-1 production.18–20 In fact, multiple factors in the diabetic milieu, such as hyperglycemia, angiotensin II (AngII), insulin-like growth factor 1 (IGF1), and stretch, enhance vascular ERK MAPK activity.21–23 In humans and animals with insulin resistance, tissues respond subnormally to insulin in increasing P13K activity, but normally in increasing MAPK activity.24,25 This imbalance between these two pathways likely contributes to a relative increase in vascular ERK MAPK activity to promote atherosclerosis.3 With the advent of “insulin sensitizers,” thiazolidinediones (TZD’s), which are ligands for the nuclear receptor peroxisome proliferator activator receptor gamma (PPARγ) and which enhance insulin-mediated glucose uptake, an important issue evolved concerning the impact of these agents on atherosclerosis.

TZDs Improve the Metabolic Syndrome
Rosiglitazone and pioglitazone are the two currently available TZDs. Both enhance the ability of insulin to transport glucose into skeletal muscle and, thus, lower circulating insulin levels.26 They are also useful for patients with type 2 diabetes...
because they decrease hepatic glucose production and prolong pancreatic \( \beta \)-cell function by preventing apoptosis of \( \beta \) cells.\(^\text{27,28}\) Both have also been shown to increase HDLC and reduce triglycerides.\(^\text{29,30}\) Rosiglitazone has been reported to increase LDLC slightly, primarily the larger buoyant form, while decreasing sd LDLC.\(^\text{31}\) Both lower blood pressure in animals, and rosiglitazone decreases blood pressure in humans with diabetes.\(^\text{32,33}\) When compared with sulfonylurea agents, both lower albumin excretion rates in patients with diabetes.\(^\text{34}\) Through actions to enhance insulin-mediated glucose uptake, through direct effects, or both, TZDs improve the metabolic, vasoactive, inflammatory, and thrombolytic milieu to potentially retard the atherosclerotic process.

### All Vascular Cells Express PPAR\( \gamma \)

In addition to its effects on circulating substances, PPAR\( \gamma \) ligands have an impact on all vascular cells relevant to the development of atherosclerosis: vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and monocyte/macrophages.\(^\text{37}–\text{40}\) PPAR\( \gamma \) is expressed in all of these cell types and, in general, has a potentially important effect of inhibiting growth and migration of vascular cells (Figure 1).\(^\text{37}–\text{43}\) These effects occur because activation of PPAR\( \gamma \) in vascular cells seems to inhibit nuclear effects of ERK MAPK signaling, such as activation of the Ets family of transcription factors, Elk-1 and Ets-1, which contribute to cell growth and movement, respectively (Figure 2).\(^\text{41,44}\) Thus, while PPAR\( \gamma \) ligands activate the P13K pathway, they tend to inhibit steps in the ERK MAPK pathway and, therefore in simplified terms, restore the imbalance in insulin signaling associated with insulin resistance. This effect has profound implication in the vessel wall.

TZDs inhibit growth and migration of VSMCs.\(^\text{37,38,41}–\text{43}\) Their antiproliferative effect is the result of cell cycle arrest in G\(_1\) associated with inhibition of phosphorylation of the retinoblastoma (Rb) gene product, which is required to release E2F, an important transcription factor regulating production of proteins required for progression to G\(_2\).\(^\text{44}\) TZDs do not affect levels of cyclins or cyclin-dependent kinases, which are necessary for G\(_1\) to S progression, but they inhibit growth factor-induced downregulation of the cyclin-dependent kinase inhibitor, p27\(^\text{kip1}\). TZDs do not transcriptionally regulate p27\(^\text{kip1}\) levels; they seem to prevent its mitogen-induced proteolytic degradation, which occurs by the process of ubiquitination and targeting to proteosomes.\(^\text{45}\)

In addition, TZDs prevent growth-factor–induced upregulation of p21\(^\text{cip1}\), which also appears to be necessary in cell cycle progression.\(^\text{41}\) Inhibition of migration also involves the ERK MAPK pathway. Pharmacologic ERK MAPK inhibitors, such as PD98059, or ERK MAPK antisense oligodeoxynucleotides inhibit migration of VSMCs induced by chemoattractants such as AngII or platelet-derived growth factor (PDGF).\(^\text{42,46,47}\) ERK MAPK phosphorylates cytosolic myosin light chain kinase (MLCK) and, at a nuclear level, enhances matrix metalloproteinase (MMP) production; both events are necessary for cell movement.\(^\text{48}\) Ets-1 transcriptionally regulates MMP expression.\(^\text{49}\) PPAR\( \gamma \) ligands do not inhibit MLCK phosphorylation, but do prevent VSMC MMP production.\(^\text{44}\) Inhibition of VSMC growth and migration by PPAR\( \gamma \) ligands in vitro translates into an in vivo alteration of neointima formation.\(^\text{42,50}\) Indeed, clinical trials are currently underway to evaluate the role of rosiglitazone and pioglitazone in prevention of coronary artery shunt restenosis.

TZDs also inhibit EC growth and migration, although the cellular mechanisms of these effects are not as well delineated as those in VSMCs.\(^\text{51}–\text{53}\) This inhibition leads to a suppression of vascular endothelial growth factor (VEGF)-induced tube formation and, ultimately, angiogenesis.\(^\text{51}–\text{53}\) In fact, direct injection of rosiglitazone into the vitreous of two ocular rat models of neovascularization, (1) hypoxia-induced retinal neovascularization, which resembles proliferative diabetic retinopathy, and (2) laser photocoagulation-induced neovascularization of the choroid, prevents angiogenesis and hemorrhage into the retina or choroid, respectively, in the two models.\(^\text{52,53}\)

PPAR\( \gamma \) is expressed in human and rodent monocyte/macrophages and in macrophage foam cells in atherosclerotic lesions.\(^\text{37}–\text{40}\) PPAR\( \gamma \) ligands inhibit migration of monocytes, which is also an ERK MAPK–dependent process.\(^\text{40,54}\) In addition, high doses of ligand in vitro inhibit vascular cell adhesion molecule (VCAM) expression in ECs leading to decreased monocyte adhesion to ECs, as well as inflammatory actions of macrophages, including their expression of interleukin (IL)-1, IL-6, tumor necrosis factor \( \alpha \) (TNF\( \alpha \)), inducible nitric oxide synthetase (iNOS), and CCR-2.\(^\text{55}–\text{58}\)

These data suggest that PPAR\( \gamma \) ligands may attenuate inflammation and, hence, atherosclerosis in the vessel wall. In sharp contrast, Tontonoz et al\(^\text{49}\) showed that oxidized LDLC increased PPAR\( \gamma \) expression in monocytes, and ligands to PPAR\( \gamma \) increased expression of CD36, leading to enhanced macrophage uptake of oxidized LDLC, thus enhancing foam
cell formation. However, subsequent investigation by the same authors and others demonstrated that activation of PPARγ induces the ABCA1 transporter, which regulates reverse cholesterol transport in macrophages.60–62 This action involves PPARγ-dependent activation of the nuclear receptor LXRα, which directly regulates expression of ABCA1.60,61

PPARγ Ligands Attenuate Early and Advanced Atherosclerosis

When animals null for the LDLC receptor (LDLR−/−) are given a high-fat or high-fructose diet, they develop hypercholesterolemia and early atherosclerotic changes, ie, fatty streak formation in their vessels.63,64 The high-fat diet induces weight gain and hyperinsulinemia, and by three months of being fed the diet, the animals become hyperglycemic.63–65 High-fructose feeding is not associated with weight gain or the development of insulin resistance in this strain.63,64 Troglitazone administered to male LDLR−/− mice on either diet attenuated lesions by 30% to 40%.66 Rosiglitazone and GW 7845 had similar effects in male LDLR−/− mice on the high-fat diet.65 None of the drugs were effective in female LDLR−/− mice.64,65 These effects were associated with evidence of decreased macrophage accumulation into the lesions, as well as decreased expression of some inflammatory markers such as TNFα.64,66 Further evidence for a role of macrophage PPARγ in attenuation of atherosclerosis was provided by the observation that replacement stem cells null for PPARγ into LDLR−/− mice who received bone marrow irradiation was associated with a 37% increase in lesion formation.62 Thus, activation of macrophage PPARγ attenuates early events in the atherosclerotic process, while loss of function accelerates early lesion formation. An important question regards the role of PPARγ in VSMCs and ECs and whether reverse cholesterol transport plays an important role in attenuation of the atherosclerotic process by PPARγ ligands.

AngII infusion markedly accelerates atherosclerosis in LDLR−/− mice and in mice null for apolipoprotein E (apoE−/−), another model of hypercholesterolemia which develops lesions even in the absence of dietary alterations.66–68 In pressor or subpressor doses, AngII promotes the formation of atherosclerotic plaques characterized by necrotic lipid cores covered by a fibrous cap and surrounded by proteoglycan matrix. Older animals develop abdominal aortic aneurysms.67 Rosiglitazone and a non-TZD PPARγ ligand, Merck L645, attenuated atherosclerosis in LDLR−/− mice administered a high-fat diet and infused with AngII for two months.68 Both PPARγ ligands reduced surface lesions by 60%.68 These effects were independent of alterations in blood pressure, circulating glucose, insulin, total cholesterol, HDLc, or triglycerides.68 However, lesions that developed despite administration of PPARγ ligands could form advanced plaques, and aneurysm formation was not impaired.68 This observation suggests that PPARγ ligands attenuate early steps in atherosclerosis formation even in the presence of AngII, but factors that contribute to complexity of lesions may not be affected. The mechanism of these effects needs further evaluation. Nevertheless, PPARγ ligands hold promise to prevent atherosclerosis in man.

Clinical Evidence of Vascular Protection by PPARγ Ligands

Troglitazone, rosiglitazone, and pioglitazone all improve EC function in humans when measured by brachial artery response to acetylcholine or when analyzed by small-vessel compliance.69 Although the improvement in EC function is a prerequisite for vascular protection, agents that improve endothelial NO activity do not necessarily improve cardiovascular morbidity and mortality.70 In addition, troglitazone has been shown to improve carotid intimal medial wall thickness in patients with diabetes in a three-month study.71 These observations likely result from effects of PPARγ ligands to 1) reduce insulin resistance and components of the insulin resistance syndrome, 2) reduce circulating factors that are associated with atherosclerosis, and 3) have a direct impact on vascular cells. Clearly, clinical trials will be necessary to determine whether activation of this important nuclear receptor will prevent both diabetes and cardiovascular disease.

References


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