Laminar Shear Stress Regulates Liver X Receptor in Vascular Endothelial Cells

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Objective—The liver X receptors (LXRs) regulate a set of genes involved in lipid metabolism and reverse cholesterol transport. We investigated the mechanism by which shear stress regulates LXR in vascular endothelial cells (ECs).

Methods and Results—Western blot showed that the protein level of LXRα and its target ABCA1 in the mouse thoracic aorta was higher than that in the aortic arch. As well, the mRNA level of LXR and its target genes ABCA1, ABCG1, ApoE, and LPL in the thoracic aorta was higher. In vitro, bovine aortic ECs were subjected to a steady laminar flow (12 dyne/cm²). The expressions of LXR and the LXR-mediated transcription were increased by laminar shear stress. Laminar flow increased LXR-ligand binding and the gene expression of sterol 27-hydroxylase (CYP27), which suggests an increased level of LXR ligand in ECs. This effect was attenuated by LXRα and CYP27 RNAi. The decrease of LXR in the aorta of PPARγ−/− mice and that of C57 mice fed with PPARγ antagonist suggest the involvement of PPARγ in the LXR induction by flow.

Conclusion—Laminar flow increases LXR function via a PPARγ-CYP27 dependent mechanism, which reveals an atheroprotective role for laminar flow exerting on endothelium. (Arterioscler Thromb Vasc Biol. 2008;28:000-000.)

Key Words: shear stress • LXR • PPARγ • endothelial cells

Shear stress resulting from blood flow plays a central role in vascular physiology and pathophysiology.1,2 Laminar shear stress in the straight parts of the arterial tree enhances vascular functioning, including the regulation of vascular tone, inhibition of cell proliferation and thrombosis, and augmentation of antiinflammatory effects. In contrast, disturbed flows with large oscillation near bifurcations and curvatures are considered proatherogenic. Thus, laminar shear stress is atheroprotective, whereas disturbed flow patterns are atheroprone. At the molecular and cellular levels, endothelial cells (ECs) have distinct mechanotransduction mechanisms responding to laminar versus disturbed flow patterns, which predispose the vessel wall to other chemical atherogenic factors.3,4 In vitro flow channel experiments have revealed the atheroprotection of steady laminar flow through promoting the expression of antioxidative and anti-inflammatory genes, antia apoptosis, and EC cycle arrest.8–10

Histochemical and biochemical study has led to the hypothesis that impaired lipid homeostasis in the vessel wall induces the accumulation of low-density lipoprotein (LDL) and its metabolic products in the subendothelial space, macrophages, and vascular smooth muscle cells, thus leading to intimal thickening and plaque formation.11 However, the mechanism by which local flow patterns causes impaired lipid homeostasis is still unclear.

Belonging to the nuclear receptor superfamily, the liver X receptors (LXRs), including LXRα and LXRβ, play central roles in the transcriptional regulation of genes that participate in reverse cholesterol transport and lipid metabolism. Synthetic LXR agonists promote cholesterol efflux and inhibit the development of atherosclerosis in mice.12–14 Administering a synthetic LXR ligand to ApoE−/− and LDLR−/− mice decreased lesion size by approximately 50%.14 The transplantation of bone marrow from LXRαβ−/− mice to ApoE−/− or LDLR−/− recipient mice resulted in a marked increase in lesion size.15 These results strongly support the notion that the LXR pathway prevents cholesterol overload. Thus, LXR agonists would be pharmacological targets for treating vascular disease associated with hyperlipidemia. Given the common atheroprotective effect of LXR and laminar shear stress, we investigated whether laminar shear stress modulates LXR in ECs in vitro and in vivo and if so, the underlying mechanism.

Materials and Methods

Cell Cultures and Flow Experiments

Human umbilical vein ECs (HUVECs) and bovine aortic ECs (BAECs) were cultured as described.15 To perform shear stress experiments, ECs were seeded on a glass slide until confluent, and the monolayer of ECs in a parallel-plate flow channel was exposed...
to a steady laminar flow at 12 dyne/cm² or 2 dyne/cm² for 12 hours. The flow system was kept under 95% air/5% CO₂ at 37°C.

**Animal Experiments**

The animal experimental protocols were approved by Peking University Institutional Animal Care and Use Committee. Male C57/BL6 or PPARγ⁺/⁻ mice were fed with standard laboratory chow and tap water ad libitum for 8 weeks. The mice received the LXR ligand TO901317 (5 mg/kg; Cayman Chemical Co) by oral gavage or a PPARγ antagonist, bisphenol A diglycidyl ether (10 mg/kg; BADGE, Sigma), by daily intraperitoneal injection. After 7 days’ treatment, animals were euthanized and the intima layer of different parts of the aorta was isolated. In some experiments, tissue from the entire aorta was homogenized. Specimens from at least 3 mice were pooled, and proteins or RNA were extracted immediately and stored at −80°C until use.

**Western Blot Analysis**

The primary antibodies used in Western blot were polyclonal anti-LXRα, LXRβ (Santa Cruz Biotech), anti-ABCA1 (Novus Biologicals), and polyclonal anti–β-actin (Biosynthesis Biotech).

**Quantitative Real-Time RT-PCR**

Total RNA was isolated from cells or aortic intima by use of RNATrip reagent (Applied). The cDNAs converted from the isolated RNA were used as templates for quantitative RT-PCR with the EVA Green fluorescent DNA stain (Biotium). The nucleotide sequences of the primers were as follows: LXRα, 5'-AAAGGAGCCCGGTTACACT-3' and 5'-AGAGGGGAAACA-GGCTCATGC-3'; LXRβ, 5'-GGAGCTGGCCATCATCCTACCC-3' and 5'-GTCTTCTAGCGACATGATCTCGATAGT-3'; ABCA1, 5'-GTGTTGCCCTCTACCTGCG-3' and 5'-ATACGGGCCGACTTTCC-5'; ATP binding cassette transporter G1 (ABC1), 5'-ACT- GCAGCATCGTGATCTGGA-3' and 3'-CGCTCTCTGATGTGTC-ACAGTG-5'; apoE, 5'-CTGTGGTTGCGCTTGTGCTG-3' and 3'-AGTTGCTGGTCATGAGTCC-5'; LXRα, 5'-AGATGGACCTACTCGTCGATGTC-3' and 5'-ACTCCGCTCTCC-5'; sterol 27-hydroxylase (CYP27), 5'-AGAGTTTGACCGCCTTCC-3'; lipoprotein lipase (LPL), 5'-AAAGGAGGAACAACTGCTGATTGGCCGAAGTTGTTGGTGCAATCT-3' and 5'-CTAGAAACATCTCCTGCGGTCATGGACATAGG-3'.

**Plasmid, Adenovirus, and Transfection**

BAECs were cotransfected with pABCA1(-928)-luc, a reporter plasmid of the hABCA1 promoter; its LXR binding element DR4, or a PPARγ binding element pABCA1-DR4m-luc; or LXREx3 TK-luc, and LXR ligandbinding plasmid of the hABCA1 promoter; its LXR binding element DR4 or a PPARγ binding element pABCA1-DR4m-luc; or LXREx3 TK-luc, and LXR ligand

**RNA Interference**

siRNA for LXRα, CYP27, or random siRNA (Invitrogen) were transfected into HUVECs by oligofectamin method (Invitrogen) for 24 hours. Then the siRNA-targeted cells were subjected to various treatments. The isolated RNA was analyzed by real-time PCR.

**Results**

**Differential Expression of LXR and Its Target Genes in Thoracic Aorta and Aortic Arch**

Blood flow in the straight part of the arterial tree is steady and laminar, whereas that in the bends and branches is disturbed. We examined first the level of LXRα and its target gene ABCA1 in different parts of the mouse aorta. The intima of the thoracic aorta and aortic arch were isolated from C57/BL6 mice treated with or without the LXR agonist TO901317. Because of the low abundance of the protein in the intima, LXRα protein was not detected in untreated aortas. Treatment with TO901317 increased the level of LXRα and ABCA1 (Figure 1A), and the expression was higher in the thoracic aorta than in the aortic arch. The mRNA level of LXRα and
ABCA1 as well as other LXR target genes, including ApoE, ABCG1, and LPL, was higher in the thoracic aorta than in the aortic arch (Figure 1B and 1C). Although treatment with TO901317 increased the level of LXRα and ABCA1 mRNA in the thoracic aorta, the mRNA level of LPL, ApoE, and ABCG1 was not further increased over that in controls. Thus, the vascular endothelium exposed to laminar flow (eg, thoracic aorta) shows an increased expression of LXRα and its target genes.

**Laminar Shear Stress Upregulates the Expression of LXRs and Their Target Genes in ECs**

We next investigated whether laminar shear stress affects the expression of LXR in cultured ECs. Confluent BAECs underwent laminar flow at 12 dynes/cm², 2 dynes/cm², or static conditions for 12 hours, and the LXRα mRNA level in the cells was assayed accordingly. Laminar shear stress at 12 dynes/cm² upregulated both LXRα and LXRβ mRNA, whereas low shear stress, similar to static conditions, had no significant effect on the level of LXRα and LXRβ mRNA (Figure 2A). These results suggest that the elevated expression of LXRs in the thoracic aorta was due, at least in part, to the exposure to the atheroprotective flow in that area. We therefore used laminar shear stress at 12 dynes/cm² to explore the effect of laminar flow on ECs and static conditions as a control in the following experiments. As compared with static conditions, laminar shear stress increased the level of LXRα and LXRβ, and ABCA1 protein (Figure 2B). As well, the level of mRNA encoding ABCG1, LPL, and ApoE in ECs increased significantly under laminar shear stress, similar to that stimulated with TO901317 (Figure 2C).

**Laminar Shear Stress Activates LXR Activity in ECs**

To investigate whether the increased expression of LXR in ECs by laminar shear stress is associated with increased LXR transcriptional activation, we transfected BAECs with 4xLXRE-Luc and pABCA1(-928)-Luc. The transfected cells were then exposed to a laminar shear stress for LXR activation assays. The LXR agonist TO901317 was used as a positive control. Laminar shear stress and TO901317 increased LXR-mediated luciferase activity by 5- and 7-fold, respectively (Figure 3A). Compared with control conditions, laminar shear stress and treatment with TO901317 increased the ABCA1 promoter-mediated luciferase activity by 2.2- and 4.8-fold (Figure 3B). Mutation of LXRE in the ABCA1 promoter (ie, ABCA1-DR4-Luc) impaired the induction by either shear stress or TO901317. To test whether laminar shear stress increased LXR transcriptional activity in a ligand-dependent manner, the plasmid CMX-GAL-hLXRα and a GAL4 reporter were cotransfected into ECs. As shown in Figure 3C, laminar shear stress increased the luciferase activity by 3.2-fold, whereas treatment with TO901317 increased the activity by 9.5-fold, which suggests that laminar shear stress may produce endogenous LXR ligand(s) in ECs.

**PPARγ Antagonist Inhibits the Activation of LXRs by Laminar Shear Stress**

PPARγ has been reported to be involved in LXRα expression and thereby stimulate ABCA1-dependent cholesterol efflux to ApoAI.19,20 Our recent study demonstrated that laminar shear stress can activate PPARγ in ECs.19 Thus, the laminar shear-stress-induced LXR might occur via a PPARγ-dependent mechanism. We included the PPARγ antagonist BADGE in shear stress experiments to investigate whether PPARγ was involved in the induction of LXRs. BADGE greatly reduced the mRNA level of shear stress–induced LXRα and LXRβ and their target genes ABCA1 and ABCG1 in BAECs (Figure 4A). To further study the role of PPARγ in LXR activation in vivo, we fed C57BL/6 mice with BADGE for 7 days and then investigated the gene expression profile in the mouse aorta. The elevated expression of LXRα, LXRβ, ABCA1, and ABCG1 in the thoracic aorta was reversed on treatment with BADGE (Figure 4B). By using PPARγ−/− mice, we reinforced the role of PPARγ in the flow-activated LXRs and their target genes. As seen in Figure 4C, the expression of LXRα, LXRβ, ABCA1, and ABCG1 in the thoracic aortas of PPARγ−/− was much lower than those in

![Figure 2. Laminar shear stress upregulates LXRs and their target genes.](image-url)
Thus, data from our in vitro and in vivo experiments suggest that atheroprotective flow-induced LXR is mediated through a PPARγ-dependent pathway.

Laminar Shear Stress Increases CYP27 mRNA Level via a PPARγ-Dependent Mechanism

High levels of CYP27 in ECs can actively catalyze cholesterol to 27-hydroxycholesterol (27-HO), an endogenous LXR ligand.21 We previously reported that 27-HO could increase the ABCA1 mRNA level in ECs,15 and others have reported that CYP27 is regulated by PPARγ.22 To further explore the mechanism by which laminar shear stress increases the level of the endogenous LXR ligand in ECs, we studied the modulation of CYP27 in ECs. The CYP27 mRNA level was increased 3.5-fold by laminar shear stress, as revealed by real-time RT-PCR (Figure 5A). However, BADGE treatment completely blocked the induction of CYP27 by laminar shear stress. As a positive control, infection ECs with an adenovirus expressing PPARγ or treating ECs with rosiglitazone also increased the CYP27 mRNA level (Figure 5B). Consistent with results from in vitro experiments, the expression of CYP27 mRNA was higher in the thoracic aorta than in the aortic arch (Figure 5C). In mice fed with BADGE, the induction of CYP27 in the thoracic aorta was abolished. This inhibitory effect of BADGE was also confirmed by the use of PPARγ-null mice.

We further knocked down the endogenous LXRα or CYP27 by siRNA and studied the consequent induction of LXR and their target genes induced by laminar shear stress. Figure 6 shows that LXRα knockdown attenuated the shear stress-induced mRNA encoding ABCA1, ABCG1, ApoE, and LPL. On the other hand, CYP27 siRNA, like LXRα siRNA downregulated ABCA1, ABCG1, ApoE, and LPL, but did not affect the expression of both LXRα and LXRβ. Thus, LXR and CYP27 synergistically regulated the expression of the LXR-target genes in ECs responding to laminar shear stress.
Discussion

Shear stress, the tangential component of hemodynamic forces acting on the vessel wall, is a major regulator of endothelial functions and vascular remodeling. Among the various flow patterns related to vessel geometry, laminar shear stress is crucial for maintaining vascular functions. Because of the protective role of LXR in atherosclerosis, we studied the effect of laminar shear stress on the activation of LXR. The major findings in this study are that (1) the expression of LXRα, LXRβ, and their target genes is higher in atheroprotective areas of mouse aorta (eg, the thoracic aorta) than atheroprone areas (eg, aortic arch); (2) compared with static conditions and low shear stress, laminar shear stress increases the expression and activation of LXRs, leading to enhanced expression of their target genes in cultured ECs; (3) the activation of LXR by shear stress is PPARγ-dependent; and (4) laminar shear stress may upregulate CYP27 through a PPARγ-dependent mechanism. CYP27, in turn, may increase the level of 27-HO, an endogenous LXR ligand.

The level of LXR in the mouse aortic arch, where ECs are exposed to disturbed flow patterns, differed from that in the thoracic aorta, where ECs experience steady laminar flow. Recently, by using microcomputed tomography and ultrasoundography, Suo et al developed a computational fluid dynamics model to map aortic flows in C57/BL6 mice. The authors used a quantum dot-based approach to correlate the expressions of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in areas where shear stress was low and unsteady. In addition, through en face staining, Cybulsky and colleagues demonstrated NF-κB activation and enhanced VCAM-1 expression in the endothelium in regions prone to disturbed flow in LDL receptor null (LDLR−/−) mice fed an atherogenic diet. Both studies demonstrated that the lesion-predisposed sites located in the lesser curvature of the aortic arch of mice. By performing Western blotting and real-time PCR, we found that the expression of LXR and its target gene ABCA1, presumably antiatherogenic, is elevated in thoracic aorta which regions are resistant to atherosclerosis. Future experi-
ments involving quantum dot or confocal microscopy would show spatial distribution patterns in different areas of arterial tree.

LXRs, including LXRα and β, are key modulators of lipid metabolism. LXRα is highly expressed in the liver and at lower levels in other tissues, whereas LXRβ is ubiquitously expressed. LXRα is also expressed in ECs, and we previously reported that native LDL/cholesterol can activate LXR, but oxidized LDL inhibits LXR activation by interrupting the 27-OH production in ECs. In the current study, we showed that LXRα and LXRβ mRNA in ECs was induced by laminar shear stress. Once activated, LXRs induce the expression of an array of genes involved in cholesterol metabolism and efflux. Ulven et al have shown that autoregulation of LXRα gene is tissue-specific, in which LXRα was upregulated by T0901317 in white adipose tissue but not in liver and muscle of C57BL6 mice. In this study, T0901317 also increases the level of LXRα and ABCA1 in the aortas of C57BL6 mice. Notably, other LXR target genes were also increased in response to the treatment of T0901317 in vitro but not in vivo. It was possibly because of the other effects of systematical administration of T0901317, because it may cause dyslipidemia and lipid accumulation in the liver. As one of the most important target genes of LXR, ABCA1 mediates the efflux of cholesterol from cells to lipoprotein in the circulation. ABCA1 expression was markedly increased in the endothelium in the straight part of the aorta. Further flow channel experiments indicated that ABCA1, as well as other target genes such as ApoE and ABCG1, were induced by laminar shear stress in ECs. Thus, one novelty of the current study is the finding that steady laminar shear stress protects the vascular endothelium, in part by upregulating LXRs, which may facilitate lipid efflux.

Several studies revealed that PPARγ upregulates LXR and laminar flow activates PPARγ in ECs. Because shear stress–activated PPARγ has been suggested to increase the production of PPARγ ligands, we treated ECs with the PPARγ inhibitor BADGE before laminar shear stress or used PPARγ−/− mice in vivo. The reduced level of the shear stress–induced LXR transcription by BADGE suggests that the upregulation of LXRs by laminar shear stress is mediated through PPARγ, which was consistent with the reduced levels of PPARγ, LXRα, and LXR-target genes in thoracic aorta of PPARγ−/− mice. Because ablation of PPARγ is lethal, heterozygous mice were therefore created for studying the function of PPARγ in different tissues. The expression of PPARγ and its target genes in vasculature of the current PPARγ−/− line was shown in Figure 4C. The possible explanations for the drastic decrease of PPARγ and its target genes in our PPARγ−/− mouse model are that the expression of PPARγ in vasculature requires 2 functional alleles. Further, PPARγ may control both LXR gene expression and ligand generation. Thus, a partial ablation of the PPARγ mice would markedly decrease the LXR-target genes such as ABCA1.

CYP27, belonging to the P450 superfamily, is an enzyme producing the LXR ligand 27-HO. Nagy and colleagues found that the human CYP27 gene is regulated by retinooids and ligands of PPAR via a PPAR-retinoic acid receptor response element in its promoter region. Induction of the expression of the enzyme results in an increased level of 27-HO and upregulation of LXR-ABCA1–mediated lipid efflux. We showed that laminar flow upregulated the CYP27 mRNA with subsequent LXR activation, which was inhibited by BADGE, a PPARγ inhibitor in cultured ECs. In vivo, CYP27 mRNA level was higher in the mouse thoracic aorta than that in aortic arch. The elevated expression of CYP27 mRNA in the thoracic aorta was abolished if animals received BADGE. Knockdown CYP27 could attenuate the effect of laminar flow, suggesting that the laminar shear stress–modulated PPARγ may be involved in the LXR activation via CYP27.

In conclusion, our results suggest a newly defined role of laminar shear stress in protecting the endothelium. Laminar flow activates LXR in ECs by a PPARγ/CYP27 pathway, which not only increases LXR expression but also enhances the LXR ligand levels. As a result, the expression of LXR target genes such as ABCA1 and ABCG1 is enhanced. These gene products actively participate in lipid efflux and increase reverse cholesterol transport and therefore are atheroprotective.

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Disclosures
None.

References


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