Peroxisome Proliferator-Activated Receptor-γ–Mediated Effects in the Vasculature

Sheng Zhong Duan, Michael G. Usher, Richard M. Mortensen

Abstract—Peroxisome proliferator-activated receptor (PPAR)-γ is a nuclear receptor and transcription factor in the steroid superfamily. PPAR-γ agonists, the thiazolidinediones, are clinically used to treat type 2 diabetes. In addition to its function in adipogenesis and increasing insulin sensitivity, PPAR-γ also plays critical roles in the vasculature. In vascular endothelial cells, PPAR-γ activation inhibits endothelial inflammation by suppressing inflammatory gene expression and therefore improves endothelial dysfunction. In vascular smooth muscle cells, PPAR-γ activation inhibits proliferation and migration and promotes apoptosis. In macrophages, PPAR-γ activation suppresses inflammation by regulating gene expression and increases cholesterol uptake and efflux. A recurring theme in many cell types is the modulation of the innate immunity system particularly through altering the activity of the nuclear factor κB. This system is likely to be even more prominent in modulating disease in vascular cells. The effects of PPAR-γ in the vascular cells translate into the beneficial function of this transcription factor in vascular disorders, including hypertension and atherosclerosis. Both human genetic studies and animal studies using transgenic mice have demonstrated the importance of PPAR-γ in these disorders. However, recent clinical studies have raised significant concerns about the cardiovascular side effects of thiazolidinediones, particularly rosiglitazone. Weighing the potential benefit and harm of PPAR-γ activation and exploring the functional mechanisms may provide a balanced view on the clinical use of these compounds and new approaches to the future therapeutics of vascular disorders associated with diabetes. (Circ Res. 2008;102:283-294.)

Key Words: PPAR-γ, vascular endothelial cells ■ vascular smooth muscle cells ■ macrophages ■ vascular disorders

Vascular complications such as atherosclerosis and hypertension are primary causes to mortality associated with diabetes and obesity. With the increasing prevalence of diabetes and obesity, vascular protection is critical to decreasing this mortality and improving public health as a whole. To accomplish this protection, one member in the nuclear receptor superfamily, peroxisome proliferator-activated receptor (PPAR)-γ, has emerged as an important player. PPAR-γ ligands, thiazolidinediones (TZDs), are clinically used for type 2 diabetes. It is clear that improving glucose and lipid homeostasis by treating diabetes or insulin resistance has beneficial effects on cardiovascular diseases.
However, the PPARs and particularly PPAR-γ have been implicated in direct actions at the vascular level.\textsuperscript{3–5} PPAR-γ is expressed in the vascular cells, and stimulation by agonists has antiinflammatory and antihypertensive effects that would be expected to be beneficial. PPAR-γ in the macrophage is also important, and interaction of macrophages with vascular cells is critical in atherosclerotic lesion progression. As a result, a new paradigm has been proposed in which PPAR-γ and other nuclear factors (NFs), most notably NF-κB, which is critical for the innate immunity in the vasculature, are seen as major controllers of susceptibility to vascular injuries and diseases and hence targets for modification of these diseases.

**Peroxisome Proliferator-Activated Receptor-γ**
PPAR-γ is a member of the nuclear hormone receptor superfamily.\textsuperscript{3} Like other nuclear receptors, it has a ligand-binding domain and a DNA-binding domain. Two major splice isoforms of PPAR-γ have been identified in mouse, PPAR-γ1 and PPAR-γ2, whereas in humans and other species, at least 2 other isoforms, PPAR-γ3 and PPAR-γ4, have also been detected.\textsuperscript{3,5,12,13} PPAR-γ3 and PPAR-γ4 encode the same protein as PPAR-γ1, which is expressed ubiquitously, including in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), macrophages, and cardiomyocytes, whereas PPAR-γ2 is mainly expressed in adipocytes.\textsuperscript{4}

TZDs, including antidiabetic drugs that are currently on the market, rosiglitazone (Avandia) and pioglitazone (Actos), are ligands of PPAR-γ.\textsuperscript{7} Natural ligands include 15-deoxy-Δ12,14-prostaglandin J2 (15-dPGJ2), 9- and 13-hydroxyoctadecadienoic acid, 12- and 15-hydroxyeicosatetraenoic acid, and nitro lipids.\textsuperscript{8–10} However, a definitive physiological endogenous ligand is yet to be identified.

PPAR-γ forms a heterodimer with another nuclear receptor retinoid X receptor (RXR)-α.\textsuperscript{3} The PPAR-γ/RXR-α heterodimers are permissive in that they can be activated by either PPAR-γ or RXR-α ligands.\textsuperscript{3} These heterodimers bind to specific DNA sequences, termed PPAR response elements (PPREs), in the regulatory regions of target genes. The PPREs are direct repeats of the consensus sequence AGGTCA separated by a single nucleotide spacer (also referred to as DR-1).\textsuperscript{3} However, it is often difficult to recognize functional PPREs. Subsequent to ligand binding, the complex undergoes conformational changes that dissociate transcriptional corepressors, including nuclear receptor corepressors and silencing mediator of retinoid and thyroid receptors, and promote association of transcriptional coactivators such as members of the steroid receptor coactivator family. These changes facilitate the recruitment of the transcriptional machinery and allow PPAR-γ to regulate the expression of a constellation of genes.\textsuperscript{3,11–14}

Alternatively, PPAR-γ interacts with other transcription factors and may not involve direct DNA binding to regulate gene transcription. For example, PPAR-γ has been shown to interact with activator protein (AP)-1, STAT (signal transducers and activators of transcription), and NF-κB, all of which are transcription factors that regulate gene expression.\textsuperscript{3,5,12,13} The proinflammatory transcription factor NF-κB plays a central role in the immune and inflammatory responses.\textsuperscript{15,16} It is the major target for PPAR-γ to suppress inflammation.\textsuperscript{17,18} NF-κB mediates the inflammatory responses in vascular cells,\textsuperscript{19} and its activation significantly contributes to the development of vascular disorders such as atherosclerosis\textsuperscript{20,21} and hypertension.\textsuperscript{22}

**PPAR-γ Activation/Inactivation in Vascular Cells**
PPAR-γ is predominantly expressed in adipocytes, where its function has been explored most extensively. However, PPAR-γ is also expressed in vascular cells, and data generated in the past 15 years have shown the great importance of this receptor in vascular biology.

**PPAR-γ Activation/Inactivation in Vascular ECs**
Major cardiovascular risk factors, including smoking, hypercholesterolemia, hypertension, and diabetes, all cause endothelial dysfunction.\textsuperscript{23} The endothelium, located at the interface between the vessel wall and blood circulation, acts as a barrier between vascular tissue and blood components and modulates traffic of cells and related compounds between them.\textsuperscript{24} ECs may be activated under the influence of a variety of stimuli, including changes in hemodynamic forces, direct drug-induced cytotoxicity, mechanical injury, and immune-mediated mechanisms. Activated ECs upregulate immune responses, including secretion of adhesion molecules (such as intercellular adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1, and E-selectin) and inflammatory cytokines/chemokines (such as interleukin [IL]-1 and tumor necrosis factor [TNF]-α).\textsuperscript{24–26} These locally expressed adhesion molecules and cytokines/chemokines activate neutrophils and generate reactive oxygen species (ROS), promoting endothelial inflammation. In this way, endothelial dysfunction integrally associates with the inflammatory process to culminate vascular injury and pose threats to normal vascular function.\textsuperscript{24}

Expression of PPAR-γ, at both mRNA and protein levels, has been detected in ECs and plasmoglobin activator inhibitor (PAI)-1 has been identified as a PPAR-γ target gene in these cells.\textsuperscript{27} So far, the function of PPAR-γ in ECs has been mostly explored using agonists including TZDs and 15-dPGJ2.

Extensive data have shown that PPAR-γ agonists reduce the activation and inflammation of ECs.\textsuperscript{28–34} Firstly, PPAR-γ agonists inhibit the expression of chemokine genes (such as interferon [IFN]-inducible protein of 10 kDa [IP-10], monokine induced by IFN-γ [Mig], and IFN-inducible T-cell α-chemoattractant [I-TAC]) in cultured human ECs (Figure 1).\textsuperscript{28} The mechanisms include inhibition on the promoter activity of the chemokine genes and direct inhibition of NF-κB activation on these promoters.\textsuperscript{28} Secondly, PPAR-γ activation by agonists or constitutive activation has been shown to inhibit the expression of proinflammatory adhesion molecules (ICAM-1, VCAM-1, E-selectin), leading to decreased adherence of monocytes to the activated EC in vitro and in vivo.\textsuperscript{29,30,32–35} Mechanistic studies have revealed the importance of NF-κB, the DNA binding of which is required for the induction of these adhesion molecules.\textsuperscript{36} PPAR-γ agonists prevent phosphorylation of NF-κB p65,\textsuperscript{32} and PPAR-γ inhibits the activities of NF-κB and AP-1 to suppress the promoter activity of these molecules (Figure 1).\textsuperscript{35}
The other possible mechanism is the inhibition of the diacylglycerol–protein kinase C signaling pathway by PPAR-γ agonists. Thirdly, TZDs have been shown to reduce production of ROS and reduce injuries and inflammation in cultured ECs (Figure 1). The inhibition on the expression of NADPH oxidase components, including nox-1, nox-2, and nox-4, explains the ROS reduction by PPAR-γ agonists. Finally, in cultured ECs, TZDs and 15-dPGJ2 suppress cytokine IFN-γ–induced expression of major histocompatibility complex class (MHC) II, which is critical in T-cell activation and the induction of immune response (Figure 1).

Imbalance between endothelium-derived nitric oxide (NO) and the endothelin-1 (ET-1) or angiotensin II (Ang II) contributes to endothelial dysfunction. One feature of endothelial dysfunction is reduced bioavailability of NO, NO-mediated vasorelaxation is not only antihypertensive but also antiatherogenic. Crossstalk exists between PPAR-γ and NO. PPAR-γ ligands enhance NO production in cultured human umbilical vein ECs through PPAR-γ–dependent mechanisms such as heat shock protein 90–endothelial NO synthase (eNOS) interaction and eNOS phosphorylation. Recent data have shown that NO can activate PPAR-γ in these ECs through a p38 mitogen-activated protein kinase (MAPK) pathway but not cGMP pathway.

On the other hand, ET-1 secreted from ECs is a potent vasoconstrictor, as well as a regulator of VSMC proliferation, and therefore involved in vascular disorders such as hypertension and atherosclerosis. PPAR-γ agonists suppress the expression and secretion of ET-1 in cultured ECs, and the mechanism involves the inhibition of AP-1 pathway. Ang II is a vasoconstrictor that increases its availability in local vascular tissue when endothelial dysfunction occurs and in turn exerts more vascular damages by activating Ang II type 1 receptor to induce oxidative stress, vasoconstriction, PAI-1 expression and thrombosis. Ang II is implicated in both hypertension and atherosclerosis. Ang II infusion in mice results in endothelial dysfunction accompanied by decreased PPAR-γ mRNA and protein expression, whereas pioglitazone and rosiglitazone both improve endothelial dysfunction induced by Ang II in rats. Interestingly, Ang II type 1 receptor blockers such as telmisartan have partial PPAR-γ agonist activity, although it requires more data from the ongoing clinical trials to determine whether the combination of telmisartan and TZDs can be more beneficial in treating vascular disorders.

In addition, a variety of PPAR-γ agonists have been shown to inhibit ECs differentiation into tube-like structures in vitro, as well as vascular endothelial growth factor (VEGF)-induced angiogenesis in vivo. The mechanisms involve the suppression of VEGF receptors and urokinase plasminogen activator expression, induction of PAI-1, and elevation of apoptosis and NO production. All these data have clearly demonstrated the importance of PPAR-γ in regulating endothelial biology (Figure 1).

PPAR-γ Activation/Inactivation in VSMCs

The proliferation and migration of VSMCs play pivotal roles in the progression of atherosclerosis and the development of restenosis after vascular interventions. In these pathological processes, injured ECs, activated platelets, and inflammatory cells produce stimulating factors, including platelet-derived growth factors (PDGF), ET-1, thrombin, fibroblast growth factor (FGF), and inflammatory cytokine IL-1, to induce the proliferation and migration of VSMCs.

PPAR-γ is present in VSMCs of normal vascular wall in both humans and rodents, as well as human atherosclerotic lesions and rat neointima induced by balloon injury of aorta. Even before being recognized as a PPAR-γ ligand, pioglitazone has been shown to inhibit rat VSMC proliferation in vitro. In addition, TZDs and 15-dPGJ2 all inhibit the proliferation and migration of cultured VSMCs from both humans and/or rodents induced by various substances including PDGF, basic FGF, thrombin, insulin, or Ang II. Furthermore, TZDs inhibit medial VSMC proliferation and migration to intima in a rat model with balloon-injury of the aorta.

Inflammatory stimuli have been considered as a key contributor to VSMC proliferation and migration and PPAR-γ activation can suppress the expression of a set of inflammatory genes. TNF-α–induced gene and protein expression of VCAM-1, monocyte chemotactic protein (MCP)-1, and fractalkine (CX3CL1) can all be inhibited by PPAR-γ activation in cultured VSMCs through inhibition on the activity of NF-κB (Figure 2). IL-1β–induced gene expression of IL-6 is inhibited by PPAR-γ activation through inhibition on the DNA binding of NF-κB and CCAAT/ enhancer-binding proteins (C/EBP), which is a transcription factor that in return upregulates the transcription and expression of PPAR-γ in cultured VSMCs and therefore enhances the antiinflammatory effect of PPAR-γ (Figure 2).

In addition, the inhibition of PPAR-γ activation on the phosphorylation of STAT-3 is a critical contributor to C/EBP downregulation (Figure 2).

Detailed mechanisms of the growth suppression on VSMCs by PPAR-γ agonists have been determined (Figure 2). One of the most critical mechanisms is cell cycle arrest. TZDs and 15-dPGJ2 have been shown to prevent G/S phase transition, which is a rate-limiting step in cell cycle, in cultured VSMCs stimulated with PDGF or insulin. This prevention is most likely because of the blockade on the
phosphorylation of retinoblastoma protein (Rb),64 a critical step downstream of cyclin/cyclin-dependent kinase (CDK) complex and a gatekeeper for the G1/S phase transition.65 TZDs and 15-dPGJ2 decrease the degradation of p27 (Kip1), an inhibitor for both CDK and phosphorylation of Rb, and thus increase its availability.66 In addition, troglitazone can directly inhibit the cyclin/CDK complex.67 By these mechanisms, the cell cycle is arrested at G1 phase, and the proliferation of VSMCs is inhibited by PPAR-γ agonists (Figure 2). The data on another CDK inhibitor, p21 (Cip1), are paradoxical in that PDGF and other mitogens upregulate p21 and PPAR-γ agonists inhibit the upregulation by blocking protein kinase C-δ in cultured VSMCs.68 This may be rationalized by the observation that p21 can also act as a positive regulator to promote cyclin/CDK complex formation and eventually G1/S phase transition.66,69 Therefore, PPAR-γ agonists may inhibit VSMC proliferation through downregulation of p21.

Another possible mechanism of the growth suppression on VSMCs by PPAR-γ agonists involves c-fos. Troglitazone inhibits c-fos expression via blockade of the MAPK pathway, while inhibiting DNA synthesis in cultured VSMCs stimulated with basic FGF.61 Rosiglitazone also inhibits c-fos gene expression and dominant-negative PPAR-γ gene transfer abolishes this effect in cultured VSMCs treated with basic FGF.70 Eukaryotic initiation factor 4E-binding protein (4EBP1) and SHIP2 (Src homology 2–containing inositol phosphorytase 2) are also likely to mediate the growth inhibition of PPAR-γ agonists.71 Both 15d-PGJ2 and rosiglitazone decrease the phosphorylation of 4E-BP1 and SHIP2 that is elevated by Ang II in VSMCs when they inhibit the DNA synthesis in vitro. However, more investigations are required to show the clear involvement of these mediators.

Recently, inhibition on telomerase activity has also been implicated as an important mechanism by PPAR-γ agonists to suppress VSMC proliferation (Figure 2).72 Both rosiglitazone and pioglitazone inhibit telomerase activity in cultured VSMCs stimulated with PDGF or insulin, most likely by inhibiting mRNA and protein expression of telomerase reverse transcriptase (TERT), the catalytic component of telomerase. Conversely, overexpression of TERT significantly attenuates the growth inhibitory effect of PPAR-γ agonists in stimulated VSMCs. Furthermore, pioglitazone inhibits telomerase activation in a mouse vascular injury model with endothelial denudation. More recently, ROS have been suggested to play a role in the effect of PPAR-γ agonists on VSMCs (Figure 2). Troglitazone inhibits low-density lipoprotein (LDL)-induced proliferation of cultured human VSMCs and the production of ROS, which is shown to contribute to VSMC proliferation.65

The mechanism of the inhibitory effect on VSMC migration by PPAR-γ agonists has been less extensively studied. Available data have shown that PPAR-γ activation inhibits VSMC invasion, 1 of the 3 steps of migration, but not the other 2 steps, attachment and locomotion.73 PPAR-γ activation suppresses the Ets-1 protein expression induced by PDGF in cultured VSMCs or by balloon injury in rat aortae. Ets-1 is a transcription factor that mediates the expression of matrix metalloproteinase (MMP) that is required for VSMC invasion.74 PPAR-γ agonists inhibit mRNA and protein expression, as well as gelatinolytic activity of MMP-9, a MMP particularly important in VSMC migration (Figure 2).55

PPAR-γ gene transfer into VSMCs induces apoptosis while inhibiting proliferation and migration in vitro and in vivo.70 This indicates that VSMC apoptosis may be antiatherogenic by countering proliferation and migration. PPAR-γ agonists have been shown to induce apoptosis of VSMCs from humans or rodents.75–79 The mechanisms include upregulation of growth arrest and DNA damage-inducible gene 45 expression,75,76 as well as p53 expression (Figure 2).76 The transforming growth factor β1/Smad2 pathway77,78 and IFN regulatory factor (IRF)-179 are also involved (Figure 2). Pioglitazone increases transforming growth factor β1 secretion and phosphorylation of Smad2 in cultured VSMCs,77,78 PPAR-γ agonists increase IRF-1 gene expression while inducing VSMC apoptosis, and conversely inhibition of IRF-1 by antisense oligonucleotide decreases the VSMC apoptosis in vitro.79 However, VSMC apoptosis can decrease the stability of atherosclerotic plaques and predispose them to rupture.80 Therefore, the inhibition on the VSMC apoptosis by PPAR-γ agonists is a double-edged sword.

**PPAR-γ Activation/Inactivation in Monocytes/Macrophages**

Functional plasticity is a hallmark of macrophages in their ability to combat widely diverse pathogens and insults. The normal evolution of an inflammatory response requires carefully coordinated recruitment of functionally distinct subclasses of macrophages, which fall within a spectrum between classically activated macrophages (M1) expressing a high level of proinflammatory cytokines and ROS and alternatively activated macrophages (M2) involved in pathogen sequestration, wound healing, and phagocytosis of apoptotic cells.81,82 Improper perturbation of this dynamic has been associated with disorders such as fibrotic diseases and thus is an important consideration to the development of therapies to disorders with an inflammatory component.

PPAR-γ has been detected in rodent macrophages,83,84 neointimal lesions,66 and macrophages of human atherosclerotic lesions.85 It has been shown to be a key regulator of M1/M2 polarization. PPAR-γ agonists reduce the secretion...
and gene expression of M1-associated proinflammatory cytokines in human monocytes including TNF-α, IL-1β, and IL-6, and inhibit macrophage activation in vitro (Figure 3). Coordinately, PPAR-γ expression and activity is enhanced by M2 differentiation and in turn is required for the upregulation of numerous markers of M2 activity including arginase-1, mannose receptor, and CD36. Moreover, PPAR-γ activation during macrophage maturation from monocytes resulted in an enhanced M2 response. However, studies demonstrating that PPAR-γ reverses cytotoxic T-lymphocyte suppression, a function of M2 macrophages, and the identification of M1- and M2-activated genes that are TZD-resistant suggest PPAR-γ is involved in regulating a specific subset of genes, rather than globally tipping the scales in the M2 direction.

At the molecular level, PPAR-γ alters macrophage function through a multitude of mechanisms. PPAR-γ undergoes a ligand and sumoylation-dependant conformational shift that allows direct binding to NF-κB and recruitment of a corepressor complex N-CoR (nuclear receptor corepressor) and subsequently suppresses transcription of NF-κB target genes such as inducible nitric oxide synthase. PPAR-γ inhibits the activity of other transcription factors such as AP-1 and STAT-1, both of which are involved in the induction of proinflammatory cytokines during M1 differentiation. Concurrently, PPAR-γ activation enhances STAT-6 activity following IL-4/13 stimulation, a signaling pathway that drives M2 differentiation. More work, however, needs to be done to determine the relative contribution of each mechanism to the effects of PPAR-γ and its dependence on the environment of macrophages. In addition, it is important to clarify which activities are dependent on PPAR-γ and which are not, given that TZDs and 15-dPGJ2 have clearly PPAR-γ-independent effects in macrophages, including regulation of the production of proinflammatory cytokines such as TNF-α and/or IL-6.

Recent studies have demonstrated that direct action of TZDs on macrophage function to be a central component to their physiologic effects. Deletion of PPAR-γ in macrophages results in reduced glucose tolerance and impaired insulin sensitivity in skeletal muscle and liver as well as enhanced weight gain and insulin resistance in high-fat-fed mice. This was associated with an increase in proinflammatory cytokine expression and reduction of ATP-binding cassette G1 expression, suggesting an impairment of reverse cholesterol transport. These studies have also demonstrated that macrophage PPAR-γ is necessary for the full insulin-sensitizing effects of TZDs and that modulation of macrophage function is important in vascular disorders.

PPAR-γ Activation/Inactivation and Vascular Disorders

The importance of PPAR-γ was originally recognized in adipogenesis and insulin sensitivity. Human genetic studies, clinical trials, as well as gain-of-function and loss-of-function studies in animal models, have proven that PPAR-γ is also a critical player in the vascular disorders such as hypertension and atherosclerosis. The beneficial impacts of PPAR-γ activation on ECs, VSMCs, and macrophages suggest favorable effects on vascular disorders.

PPAR-γ Activation/Inactivation and Hypertension

Hypertension is a common complication of diabetes. These 2 chronic diseases often coexist and exacerbate each other. The prevalence of hypertension in diabetic patients is approximately twice that in nondiabetics. Conversely, the chance of developing type 2 diabetes in hypertensive individuals is almost 2.5 times as that in normotensive individuals. PPAR-γ is expressed in both vascular ECs and VSMCs, making it possible that this receptor plays roles in regulating vascular tone and blood pressure. Indeed, PPAR-γ agonists, including TZDs, lower blood pressure in diabetic patients and animal models, at least partially independent of their insulin-sensitizing effects, although this blood pressure-lowering effect is much more moderate in human patients than in animal models. Furthermore, rosiglitazone decreases blood
pressure in hypertensive transgenic mice that express both human renin and human angiotensinogen transgenes. Although not changing gene expression of important blood pressure regulators such as eNOS, angiotensin 1 receptors, and preproendothelin-1, rosiglitazone improves the impaired relaxation of carotid arteries to acetylcholine in these mice. Therefore, the blood pressure–lowering effect of rosiglitazone may be because of its direct impact on blood vessels.

PPAR-γ Pro12Ala mutation in humans is associated with lower diastolic blood pressure, which is likely independent of metabolic impact of this mutation. In contrast, dominant-negative loss-of-function mutations Pro495Leu, Val318Met, Phe388Leu, and Arg425Cys are all associated with hypertension, although it has not been determined whether the high blood pressure is because of the metabolic consequences such as insulin resistance and lipodystrophy seen in these mutations.

In mice harboring the same dominant-negative mutation of PPAR-γ (Pro467Leu) as seen in humans, hypertension and fat redistribution but not insulin resistance and lipodystrophy have been reported. This suggests that the impact of PPAR-γ on blood pressure can be separable from its influence on metabolism, although another line of dominant-negative PPAR-γ mutant mice (Leu466Ala) have hypertension (female only) as well as insulin resistance.

The mutation studies in both humans and animals have provided significant insights regarding the function of PPAR-γ in blood pressure regulation. However, it is worthwhile to point out that dominant-negative mutants of PPAR-γ can act promiscuously to inhibit activities of all 3 wild-type PPARs, PPAR-α, PPAR-δ, and PPAR-γ. Therefore, it is not conclusive that these mutants mentioned above strictly act by altering only PPAR-γ activity, and it is critical to use other approaches such as analyzing knockout phenotypes of PPAR-γ.

A generalized PPAR-γ knockout mouse model was created to circumvent the embryonic lethality of the germline PPAR-γ knockout. Given that PPAR-γ agonists lower blood pressure, it is surprising that these mice are hypotensive, while having lipodystrophy and insulin resistance, as expected. The generalized PPAR-γ knockout mice are more sensitive to endothelial-dependent relaxation to muscarinic stimulation and have decreased contraction to α-adrenergic agents. These vascular defects are likely significant contributors to the hypotension, although detailed mechanisms and other possible contributors need to be explored further. It is hypothesized that at baseline PPAR-γ suppresses gene expression in vascular cells to contribute to the maintenance of normal blood pressure and that both agonists and knockout can relieve the suppression and lead to lower blood pressure.

Another possible target for PPAR-γ to regulate blood pressure is the kidney. However, collecting duct–specific PPAR-γ knockout mice have normal blood pressure, indicating that PPAR-γ deficiency in the kidney is unlikely a contributor to the hypotension phenotype seen in the generalized PPAR-γ knockout mice.

As discussed in the early parts of this review, PPAR-γ activation in ECs improves endothelial function and in VSMCs inhibits their proliferation (Figure 4), both of which may exert the beneficial effects on blood pressure regulation. It is important to determine whether PPAR-γ in ECs, VSMCs, or both is required to maintain normal blood pressure. Endothelium-specific PPAR-γ knockout mice have normal blood pressure at baseline but when fed with high-fat diet, have higher blood pressure than littermate controls. This hypertension induced by high-fat diet was not affected by rosiglitazone treatment, suggesting that endothelial PPAR-γ is required for the blood pressure–lowering effect of TZDs. The role of smooth muscle PPAR-γ in maintaining normal blood pressure at baseline and whether smooth muscle PPAR-γ is required for TZDs to lower blood pressure need to be investigated further.

**PPAR-γ Activation/Inactivation and Atherosclerosis**

Atherosclerosis is the primary cause of mortality in patients with diabetes and underlies ~50% of all deaths in westernized countries. Insulin resistance and associated glucose intolerance, dyslipidemia, hypertension, increased oxidation, thrombosis, and vascular inflammation are all risk factors to atherosclerosis. These risk factors may cause endothelial dysfunction, leading to changes in endothelium. These changes include increased endothelial permeability to lipoproteins and other plasma constituents, upregulation of cell adhesion molecules, and migration of monocytes into the vascular wall. These changes allow LDL to enter the vascular wall and become oxidized (ox)-LDL. The monocytes migrating into the vascular wall differentiate to macrophages and take up ox-LDL, leading to foam cell formation. At the same time, these changes allow vascular inflammation to escalate and further exacerbate endothelial dysfunction. VSMCs proliferate and migrate from the media into the intima of the vascular wall, a hallmark of atherosclerotic lesions. Unstable lesions may rupture and cause thrombosis, a life-threatening condition.

As mentioned above, PPAR-γ is not only expressed in macrophages, ECs, and smooth muscle cells in normal vasculature but also in atherosclerotic lesions. PPAR-γ activation was first hypothesized to be proatherogenic, because the PPAR-γ agonists troglitazone or 15-dPGJ2 and the RXR agonist LG268 together were found to increase ox-LDL uptake dramatically in monocytic leukemia cell line THP-1 primarily through scavenger receptor CD36. Moreover, PPAR-γ can be activated by ox-LDL and its metabolites to upregulate CD36, further enhancing ox-LDL uptake by these cells. This positive feedback loop may promote foam cell formation, a key step in the development of atherosclerosis. However, troglitazone does not alter ox-LDL uptake and degradation in wild-type embryonic stem cell–derived macrophages, even though the expression of CD36 is increased and conversely, PPAR-γ–deficient macrophages have suppressed CD36 expression and ox-LDL uptake. The inhibitory effect of PPAR-γ activation on macrophage scavenger receptor class A, another receptor important for ox-LDL uptake, is likely to explain this result. Subsequent conflicting data from these two studies may be because of the differences in cell lines and incubation time.
data support an antiatherogenic role of PPAR-γ activation, which increases cholesterol efflux from macrophages by inducing both liver X receptor-α and ATP-binding cassette A1 expression. A unifying model has been put forward. PPAR-γ activation reduces the accumulation of atherogenic ox-LDL in the vascular wall by enhancing both uptake into and efflux out from macrophages (Figure 3).

PPAR-γ agonists have been found to reduce atherosclerosis or intimal to medial ratio in human patients and animal models that include LDL receptor–null mice and apolipoprotein E–null mice, although it has been demonstrated that these antiatherogenic effects can be highly sex-specific and PPAR-γ agonists are only effective in male but not female LDL receptor–null mice. In vivo gene transfer of PPAR-γ into rat artery also inhibits smooth muscle proliferation, induces apoptosis, and reduce neointima formation after balloon injury. Using a mouse model that are both atherosclerotic and insulin-insufficient, it has been demonstrated that these antiatherogenic effects can be independent of their beneficial effects on metabolism. The protections provided by PPAR-γ activation on endothelial function, VSMC proliferation and migration, and macrophage cytokine production as well as cholesterol efflux (Figure 4), likely contribute to these beneficial effects in atherosclerosis.

Pro12Ala and C161IT mutations of PPAR-γ in humans are associated with lower incidence of coronary artery disease that closely relates to atherosclerosis independently of metabolic changes. This seems to disagree with the beneficial roles of PPAR-γ activation given that these 2 mutations have been considered as loss-of-function. However, the characterization of these PPAR-γ mutations as loss-of-function was performed in vitro and function status of these mutations in humans remains to be established.

PPAR-γ deficiency in macrophages has proven to be atherogenic in animals. Consistent with the previous finding that PPAR-γ activation controls cholesterol efflux in macrophages, PPAR-γ deficiency in macrophages specifically leads to reduced cholesterol efflux, accompanied by decreased expression of lipoprotein lipase, CD36, liver X receptor-α, and ABCG1. Under the challenge with atherogenic diets, bone marrow transplantation from macrophage-specific PPAR-γ–deficient mice to LDL receptor–null mice or C57BL/6 mice resulted in significantly larger atherosclerotic lesions than wild-type bone barrow was used. A similar result was observed when bone marrow from PPAR-γ–deficient chimeric mice was used. These data further suggest that macrophage PPAR-γ is antiatherosclerotic and that CC chemokine receptor 2 may act as a modulator.

Recent data have shown that macrophage-specific PPAR-γ deficiency impairs alternative macrophage activation, which is beneficial in regulating nutrient and metabolism homeostasis. Currently being explored, more significant roles of PPAR-γ in alternative macrophage activation relating to atherosclerosis may be revealed. In particular, macrophage phenotypes are a spectrum between M1 and M2 activation. PPAR-γ agonists appear to affect many but not all genes related to M2 phenotype, suggesting that they are not simply tipping the scale toward M2 differentiation.

The importance of endothelial dysfunction, as well as VSMC proliferation and migration, in atherosclerosis has been well recognized. The beneficial effects of TZDs on the function of ECs and VSMCs suggest important roles of endothelial PPAR-γ and VSMC PPAR-γ in the development of atherosclerosis. However, the impact of endothelial PPAR-γ deficiency has not been reported in the existing endothelium-specific PPAR-γ knockout mice and generalized PPAR-γ knockout mice. VSMC-specific PPAR-γ knockout mice are yet to be developed.

**Impact of PPAR-γ Activation-Caused Metabolic Changes in the Vasculature**

High levels of circulating nonesterified fatty acids and triglycerides not only correlate with insulin resistance but also have direct impacts on the vascular wall such as affecting the production of macrophage lipoprotein lipase that is involved in the pathogenesis of atherosclerosis. PPAR-γ agonists can decrease circulating nonesterified fatty acids and triglycerides and therefore decrease the risk for vascular complications.

Epidemiological studies have shown that hyperinsulinemia, a marker for insulin resistance, is an independent predictor of coronary artery disease, as well as an important contributor to hypertension associated with diabetes. Decreased fasting insulin level and improved insulin sensitivity through interventions including PPAR-γ activation are critical for type 2 diabetes and may decrease the risk for vascular complications.

In addition, PPAR-γ activation can modulate the endocrine function of adipocytes by altering gene expression of key molecules such as leptin, adiponectin, resistin, PAI-1, IL-6, and TNF-α. The ability of TZDs to increase levels of adiponectin may contribute to the beneficial effects of PPAR-γ activation in the vasculature, because adiponectin levels are negatively associated with coronary artery disease and hypertension. TZDs act on adipocytes to decrease the expression and secretion of PAI-1, which is prothrombotic. TZDs also repress adipocyte expression of IL-6 and TNF-α, both of which are associated with vascular inflammation and therefore contribute to decreased risk for vascular disorders.

**PPAR-γ and Vascular Side Effects of TZDs**

Despite the beneficial effects of TZDs have in vascular disorders, these compounds may have some serious side effects in the vasculature. One cardiovascular side effect that seems to be a class effect is congestive heart failure, a major hurdle to the clinical use of TZDs. This has been further
confirmed by recent findings. Both rosiglitazone and pioglitazone increase congestive heart failure significantly. Fluid retention with TZD treatment remains to be the plausible cause of heart failure, and activation of PPAR-γ in the kidney is the mechanism because collecting duct-specific PPAR-γ knockout blocks the effect of TZD on fluid retention and mRNA expression of the sodium channel ENaC-γ. The PROactive study has shown significantly increased incidence of leg revascularization by pioglitazone. It is uncertain whether this is a PPAR-γ-mediated or an “off-target” effect, and further investigation is needed.

Recently, rosiglitazone treatment has been linked to increased risk in myocardial infarction and death from cardiovascular causes, although the latter only has a borderline significance statistically. More recently, another study has shown significantly increased incidence of leg revascularization by pioglitazone. The PROactive study has shown significantly increased incidence of leg revascularization by pioglitazone. It is uncertain whether this is a PPAR-γ-mediated or an “off-target” effect, and further investigation is needed.

Recently, rosiglitazone treatment has been linked to increased risk in myocardial infarction and death from cardiovascular causes, although the latter only has a borderline significance statistically. More recently, another study has confirmed that rosiglitazone treatment is associated with increased incidence of myocardial infarction but not a significant change in the risk of cardiovascular death. Of note, the conclusions of these studies were reached using metaanalysis, a methodology that has its strengths and a number of limitations. In particular, these studies had limited access to the original data. Furthermore, the findings were not confirmed by an interim analysis from a prospective study that has been designed to investigate rosiglitazone. Even though the increased risk in myocardial infarction is still controversial, these reports have brought the cardiovascular safety of this drug to the attention of many people, from clinicians to patients to Food and Drug Administration officials. These concerns are not surprising, because diabetic patients are already at higher risk for cardiovascular diseases and millions of rosiglitazone prescriptions have been dispensed annually.

However, unlike congestive heart failure, myocardial infarction does not seem to be a class effect. The PROactive study has shown that pioglitazone, another member in the TZD class of drugs, significantly decreases the occurrence of all-cause mortality, nonfatal myocardial infarction, and stroke. In patients with type 2 diabetes, these are the main components of the secondary end point. However, the reduction is not statistically significant for the primary end point (defined as the time from randomization to occurrence of a new macrovascular event or death). A more recent study using data from 19 trials, including the PROactive study, finds that pioglitazone reduces components of the primary end point (death from cardiovascular cause, heart attack, or stroke) in diabetic populations. Again, this later study used metaanalysis, a methodology that has its limitations.

Pioglitazone has more beneficial effects on lipid profile than rosiglitazone, and this may explain, at least in part, the different cardiovascular influence that rosiglitazone and pioglitazone have. However, more mechanistic studies are required to understand the causes of the differences between these 2 drugs. It is also important to further define the vascular changes as well as myocardial changes brought by rosiglitazone. More importantly, is PPAR-γ mediating these side effects of rosiglitazone? Given the variety of beneficial effects that PPAR-γ activation has in the vascular cells and vascular disorders, as discussed in the previous sections, and the data showing that pioglitazone and rosiglitazone have opposite effects in these studies of cardiac risks, non-PPAR-γ-mediated pathways are suggestive. However, no mechanism has been described and more research is needed.

Conclusions

PPAR-γ has been recognized as an important regulator in endothelial biology, vascular smooth muscle function, and macrophage function. Regulation of the innate immunity system through NF-κB is emerging as a critical function of PPAR-γ activation. Alteration of macrophage differentiation and emerging effects on vascular cells dramatically modify the pathophysiology in cardiovascular disease. Although work in experimental models has overwhelmingly shown beneficial effects of TZDs on intermediate phenotypes, the end result in humans remains in question. Recent differences among TZDs in cardiac risk raise the possibility of off-target or specific agonist effects of TZDs on PPAR-γ. Molecular studies on these effects will likely reveal differences in action of TZDs and clarify the role of PPAR-γ in mediating these effects.

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Disclosures

None.

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Peroxisome Proliferator-Activated Receptor-γ–Mediated Effects in the Vasculature
Sheng Zhong Duan, Michael G. Usher and Richard M. Mortensen

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