Peroxisome Proliferator-Activated Receptors and Atherogenesis: Regulators of Gene Expression in Vascular Cells

Nikolaus Marx, Hélène Duez, Jean-Charles Fruchart, Bart Staels

Abstract—A large body of data gathered over the past couple of years has identified the peroxisome proliferator-activated receptors (PPARs) as transcription factors exerting modulatory actions in vascular cells. PPARs, which belong to the nuclear receptor family of ligand-activated transcription factors, were originally described as gene regulators of various metabolic pathways. Although the PPARα, γ, and β/δ subtypes are 60% to 80% homologous in their ligand- and DNA-binding domains, significant differences in ligand and target gene specificities are observed. PPARα is activated by polyunsaturated fatty acids and oxidized derivatives and by lipid-modifying drugs of the fibrate family, including fenofibrate or gemfibrozil. PPARα controls expression of genes implicated in lipid metabolism. PPARγ, in contrast, is a key regulator of glucose homeostasis and adipogenesis. Ligands of PPARγ include naturally occurring FA derivatives, such as hydroxyoctadecadienoic acids (HODEs), prostaglandin derivatives such as 15-deoxyΔ12,14-prostaglandin J2, and glitazones, insulin-sensitizing drugs presently used to treat patients with type 2 diabetes. Ligands for PPARβ/δ are polyunsaturated fatty acids, prostaglandins, and synthetic compounds, some of which are presently in clinical development. PPARβ/δ stimulates fatty acid oxidation predominantly acting in muscle. All PPARs are expressed in vascular cells, where they exhibit antiinflammatory and antithromogenic properties. In addition, studies in various animal models as well as clinical data suggest that PPARα and PPARγ activators can modulate atherogenesis in vivo. At present, no data are available relating to possible effects of PPARβ/δ agonists on atherogenesis. Given the widespread use of PPARα and PPARγ agonists in patients at high risk for cardiovascular disease, the understanding of their function in the vasculature is not only of basic interest but also has important clinical implications. This review will focus on the role of PPARs in the vasculature and summarize the present understanding of their effects on atherogenesis and its cardiovascular complications. (Circ Res. 2004;94:1168-1178.)

Key Words: peroxisome proliferator-activated receptors ▪ vascular cells ▪ arteriosclerosis ▪ diabetes ▪ lipid metabolism

Peroxisome proliferator-activated receptors (PPARs), originally cloned in an attempt to identify the molecular mediators of peroxisome proliferation in the liver of rodents,1 are ligand-activated transcription factors belonging to the nuclear receptor superfamily, which also includes the steroid and thyroid hormone receptors. Upon activation by their ligands, PPARs form heterodimers with the nuclear receptor RXR and bind to specific PPAR response elements (PPREs) in the promoter region of their target genes. These PPREs consist of a direct repetition of the consensus AGGTCA half site spaced by one or two nucleotides (DR1 or DR2) (Figure 1). Initial work considered these PPREs canonical for each PPAR-regulated gene, but subsequently it became clear that PPREs demonstrate a high variability, thus allowing fine-
tuned specific modulation of gene expression. PPARs can also repress gene expression in a DNA-binding–independent manner by interfering with other signaling pathways as well as in a DNA binding–dependent way through the recruitment of corepressors to unliganded PPARs.\(^\text{3,4}\)

The PPAR family consists of three members, \(\alpha\), \(\gamma\), and \(\beta/\delta\), which share \(\sim 60\% - 80\%\) homology in their ligand- and DNA-binding domains. Although presently there are no proven pathways for endogenous ligands in vivo, all PPARs are activated by fatty acids (FAs). However, subtle differences in ligand and target gene specificity exist between the PPAR isoforms. PPAR\(\alpha\) can be activated by certain polyunsaturated FAs, including DHA and EPA, by oxidized phospholipids, by lipoprotein lipolytic products,\(^\text{5}\) and by fibrates, such as fenofibrate and gemfibrozil.\(^\text{6,7}\) Fibrates are clinically used to treat patients with lipid disorders and have been shown to reduce cardiovascular mortality. PPAR\(\alpha\) regulates genes that are involved in lipid and lipoprotein metabolism (Figure 2A).

Natural ligands for PPAR\(\gamma\) are the prostaglandin D2 derivative 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(_2\) (15d-PGJ\(_2\)) and forms of oxidized linoleic acid, 9- and 13(S)-HODE, and although these endogenous ligands can activate PPAR\(\gamma\) in vitro, presently there are no proven pathways in vivo. Synthetic ligands for PPAR\(\gamma\) include the antidiabetic thiazolidinediones (glitazones), such as troglitazone, rosiglitazone, and pioglitazone.\(^\text{8–10}\) These insulin-sensitizing drugs reduce peripheral insulin resistance and thus lower blood glucose levels in patients with type 2 diabetes. Troglitazone has been the first agent of this class on the market but was withdrawn because of liver toxicity. The presently available agents, rosiglitazone and pioglitazone, do not exhibit such undesirable side effects. The underlying mechanisms of glitazone-mediated improvement of insulin resistance are not completely understood but likely occur via activation of PPAR\(\gamma\) in adipose tissue, where it is a crucial regulator of adipogenesis.\(^\text{11}\) The induction of fat cell differentiation from large insulin-resistant adipocytes to smaller, more insulin-sensitive cells seems to be of particular importance. As a consequence of PPAR\(\gamma\) activation, there is a reduced release of free FAs and insulin resistance–mediating adipokines, such as tumor necrosis factor (TNF)-\(\alpha\), leptin, or resistin, and an increased production of the antiatherogenic, antidiabetic adipokine adiponectin. These changes are thought to lead to improved insulin sensitivity in liver and skeletal muscle (Figure 2B). In addition, although studies using muscle-specific PPAR\(\gamma\)-deficient mice yielded controversial results with respect to the response to glitazone treatment, these mice exhibit basal insulin resistance, suggesting a role for PPAR\(\gamma\) also in muscle.\(^\text{12,13}\)

The recent development of high-affinity synthetic agonists and genetically modified animal models for PPAR\(\beta/\delta\) has helped to start elucidating its (patho)physiological function. As PPAR\(\alpha\), PPAR\(\beta/\delta\) plays a role in lipid metabolism by stimulating FA oxidation in heart and skeletal muscle cells.\(^\text{14}\) PPAR\(\beta/\delta\) agonist treatment reversed the diet-induced obesity and obese Rhesus monkeys.\(^\text{16}\) Interestingly, PPAR\(\beta/\delta\) overexpression reversed the obese phenotype in db/db mice,\(^\text{17}\) and PPAR\(\beta/\delta\) agonist treatment reversed the diet-induced obesity and insulin resistance in mice.\(^\text{18}\) Thus, PPAR\(\beta/\delta\) has been proposed as a pharmacological target for the treatment of obesity, insulin resistance, and dyslipidemia. Such effects may contribute to potential vascular antiatherogenic effects of such compounds (Figure 2C).

### PPAR\(\alpha\) and Atherogenesis

#### Cellular Actions
Several research teams examined the role of PPAR\(\alpha\) in vascular biology. Based on the findings that endothelial cells (ECs),\(^\text{19,20}\) vascular smooth muscle cells (VSMCs),\(^\text{21,22}\) monocytes/macrophages,\(^\text{23,24}\) and T cells\(^\text{25}\) all express PPAR\(\alpha\), it is clear that PPAR\(\alpha\) activators act on a variety of
regulation of VCAM-1 expression by PPAR

Leukocytes, mainly monocytes and T cells, are attracted to sites of developing lesions by chemotactic pro-adhesion. Leukocytes, mainly monocytes and T cells, are involved in leukocyte recruitment and cell processes involved in developing lesions. VCAM-1, a monocyte-specific chemokine,28 regulates the expression of genes involved in VLDL synthesis, lipolysis, and HDL metabolism. PPARγ activates its regulatory effects on NO release and ET-1 expression together, PPARγ activators may counterbalance endothelial dysfunctions at different steps.

In VSMCs, PPARα activators modify inflammatory VSMC activation by inhibiting IL-1–induced production of IL-6 and prostaglandins and by reducing the expression of cyclooxygenase-2 (COX-2).21

The role of PPARα in monocytes and macrophages has been examined with respect to its effects on foam cell formation and lipid metabolism as well as its antiinflammatory properties. After adhesion to the endothelium, monocytes enter the subendothelial space, where they take up cholesterol from trapped, modified LDL to be transformed into foam cells. PPARα is present in monocytes, and its expression is upregulated during differentiation into macrophages,34 where it may control lipid homeostasis. PPARαactivators induce the expression of the HDL receptor CLA-1/SRB-I and ABCA1, a transporter controlling apolipoprotein (apo) A-I–mediated cholesterol efflux.35,36 Because this receptor is involved in cholesterol efflux, PPARα may have a beneficial effect on the regression of fatty streaks by promoting removal of unesterified cholesterol from macrophages. Moreover, PPARα ligands downregulate the expression of the apoB48-remnant receptor in differentiated macrophages and reduce the uptake of glycated LDL and triglyceride (TG)-rich remnant lipoproteins.37,38 This may be particularly important in patients with diabetes and the metabolic syndrome. Conflicting results exist on the role of PPARα on expression and activation of LPL, with data demonstrating a decreased secretion and activity of LPL39 on treatment with PPARα ligands and other studies reporting that PPARα may upregulate LPL expression in macrophages.39

In this context,
Ziouzenkova et al. have shown that lipolysis of triglyceride-rich lipoproteins generates PPAR ligands, suggesting a potentially important link between lipoprotein metabolism and distal PPAR activation and inflammatory response. Moreover, PPARs regulate macrophage intracellular cholesterol metabolism and decreases the ratio of intracellular cholesterol ester to free cholesterol by reducing acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT-1) activity. 40 Together, these data support an essential role for PPAR activation in macrophage transitions by increasing the macrophage-free cholesterol pool available for cholesterol efflux and subsequent reverse transport while decreasing macrophage lipid accumulation and ensuing foam-cell formation.

During atherogenesis, monocyte/macrophage activation is of particular importance in the formation of advanced lesions. At this stage, the atherosclerotic lesion is characterized by the presence of a large necrotic lipid core, which is covered by a thin fibrous cap. Thinning of the fibrous cap renders the plaque more vulnerable, thus increasing the risk of plaque rupture with subsequent thrombus formation with the clinical correlate of an acute coronary syndrome (ACS). Monocyte/macrophages accumulate in the shoulder region of advanced plaques and their presence largely contributes to plaque rupture and the development of ACS. Besides releasing matrix-degrading enzymes, these cells exhibit procoagulant activity by expressing tissue factor (TF) on their surface. 41 PPAR agonists reduce TF and matrix metalloproteinase (MMP) expression in monocytes and macrophages, thus potentially modulating the stability and thrombogenicity of atherosclerotic lesions. 23,24,42 Besides these effects, PPAR activation in combination with TNF-α and IFN-γ may promote macrophage apoptosis. 41 If properly controlled, such induced apoptosis could contribute to the stabilization of atherosclerotic lesions by eliminating a source of inflammatory cytokines and matrix degrading enzymes, although alternative, less-beneficial scenarios could also be envisaged.

T lymphocytes contribute to the vascular inflammatory response during initial atherogenesis. T lymphocytes, mainly CD4+ positive cells, enter the vessel wall as naive Th0-cells and differentiate in the subendothelium to Th1-cells under the influence of certain antigens like oxidized LDL. 43 Th1-cells release a variety of proinflammatory cytokines, such as IFN-γ, TNF-α, or IL-2, which then stimulate other cells in the vessel wall. Activators of PPARs and PPARγ might limit this deleterious Th1-cytokines, suggesting a modulating function of PPARs at a nodal point of vessel wall inflammation. 25

Preclinical/Clinical Data

In clinical trials, fibrates have been shown to reduce the progression of coronary atherosclerosis in the Bezafibrate Coronary Atherosclerosis Intervention Trial, 44 Diabetes Atherosclerosis Intervention Study, 45 and Lopid Coronary Angiography Trial 46 and to decrease the incidence of coronary heart disease in the Helsinki Heart Study 47 and in Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT). 48 Moreover, genetic studies on PPAR polymorphisms suggest an association between PPAR variants and the risk of coronary atherosclerosis and ischemic heart disease. 49 Treatment of patients with type 2 diabetes with different fibrates (eg, ciprofibrate, gemfibrozil, and fenofibrate) results in improved postischemic flow-mediated dilatation of the brachial artery. 50–52 A NO-mediated vascular response. In fibrate-treated non-diabetic subjects with dyslipidemia 53 or coronary disease, 54 similar improvements in endothelial function have also been observed in other vascular beds. Assessment of an antiatherogenic activity of PPAR agonists in rodent models of atherosclerosis is hampered by the fact that (1) rodents develop a proinflammatory peroxisome proliferative response in the liver and (2) classically used animal models such as the LDL-receptor or apoE-deficient mouse display an aberrant hyperlipidemic response to these hypolipidemic drugs. Nevertheless, a few studies reported on the effect of PPAR activation on atherosclerosis development in vivo in mice. PPAR-deficient mice crossed with apoE-deficient mice exhibited less insulin resistance and demonstrated reduced atherosclerosis compared with their PPAR+/−/apoE−/− littermates. 55 In addition, ciprofibrate treatment was reported to markedly increase plasma levels of atherogenic lipoproteins in apoE-deficient mice and aggravate atherosclerosis development. 56 On the other hand, fenofibrate administration to Western diet–fed, apoE-deficient mice resulted in decreased atherogenesis in the descending aorta but not in the aortic sinus. Because there were no major changes in plasma lipids, a direct vascular effect of PPAR seemed to be implicated. 57 Most relevant to the human situation, fenofibrate treatment of apoE2 knock-in mice, a mouse model of mixed dyslipidemia, resulted in significantly reduced atherosclerotic lesion size in the aortic sinus (A. Tailleux, G. Torpier, H. Mezdour, P.J.-C. Fruchart, B. Staels, and C. Fievet, unpublished observation, 2004). Therefore, it appears that in association with a reduction in plasma TG and non−HDL-C and an increase in HDL-C, both lipid-dependent and direct vascular effects of PPAR may contribute to its actions on atherosclerosis development in vivo. Although the data on the actions of PPAR agonists in rodent models are not entirely clear, the human studies conducted so far suggest that fibrates will prevent coronary atherosclerosis progression and coronary heart disease events, especially in populations with the proinflammatory conditions of diabetes (Diabetes Atherosclerosis Intervention Study and VA-HIT) and insulin resistance (VA-HIT).

The effects of PPAR activation on cardiac function have recently also been examined in animal models of cardiovascular disease (eg, ischemic injury and ventricular hypertrophy). Left ventricular hypertrophy (LVH) occurs after prolonged pressure overload related to physiological (exercise) or pathological (hypertension) stimuli and may result in contractile dysfunction and ultimately heart failure. In the hypertrophied heart, PPAR expression and activity are reduced, leading to altered substrate utilization. 58,59 This downregulation of PPARs signaling appears essential to preserve heart function against pressure overload. 60 Conversely, cardiac PPAR overexpression led to lipid accumulation and ventricular dysfunction, a phenotype further enhanced in the diabetic heart. 61,62 However, it is possible that the systemic lipid-lowering actions of PPAR agonists...
leading to reduced TG delivery to extrahepatic tissues (eg, cardiac muscle) might counterbalance these alterations in cardiac PPARα signaling in patients with diabetes.62 A recent genetic study showed an association between polymorphisms in the PPARα gene and the extent of LVH in response to training and hypertension, demonstrating a link between PPARα and LVH also in humans.63

If prolonged, left ventricular dilatation may result in cardiomyocyte hypertrophy (referred to as vascular remodeling) and interstitial fibrosis of the myocardium, progression to heart failure, and, eventually, sudden death. Interestingly, PPARα agonists decrease endothelin–1–induced myocyte hypertrophy in vitro64 and prevent cardiac remodeling and interstitial fibrosis in rat models of hypertension.65,66 Furthermore, PPARα agonists reduce infarct size after ischemia/reperfusion injury and improve contractile recovery as well as endothelial function in vivo.67 Interestingly, PPARα-deficient mice are more susceptible to ischemic damages compared with wild-type mice.68 Whether these beneficial effects of PPARα activation on heart function are attributable to its effects on lipid metabolism or the control of vascular inflammatory response, vascular tone, and redox balance69 is presently unknown.

**PPARγ and Atherogenesis**

**Cellular Actions**

PPARγ is also expressed in ECs, VSMCs, macrophages, and T cells.70–73 In vitro experiments using human saphenous vein ECs indicated that activation of PPARγ, but not PPARα, inhibits IFN-γ–induced expression of IP-10, Mig, and I-TAC, with a subsequent decrease in lymphocyte chemotaxis.28 Inhibition of IP-10, Mig, and I-TAC release from human ECs by PPARγ activators might therefore mainly limit the recruitment of activated T cells. Similar to PPARα agonists, PPARγ may act as a vasorelaxant, as evidenced by the inhibition of spontaneous and insulin-induced expression of ET-1 in ECs85 and by enhancing the release of NO from these cells.74 In hypertensive rats, glitazones diminished hypertension progression and prevented vascular remodeling. In these animals, glitazone treatment decreased ET-1 production and blunted production of oxygen free radicals.66 Controversial data exist on the role of PPARγ agonists in endothelial apoptosis. 15d-PGJ2 and ciglitazone have been shown to induce apoptosis,75 whereas a recent study suggests protective, antiapoptotic effects of glitazones in rat ECs.76 The nature of the activators used in these studies may explain this discrepancy, especially given that some of their effects may be PPARγ independent. In addition, the expression level of PPARγ may vary in cells from different vascular beds and may also depend on the proliferative status of these cells,77 which may result in changeable levels of susceptibility toward PPARγ agonists.

Other studies have examined the role of PPARγ in angiogenesis and the promotion of growth factor expression. Xin et al78 demonstrated that PPARγ agonists inhibit VEGF receptor 1 and 2 expression and reduce endothelial tube formation in vitro and provided evidence that PPARγ agonists might limit angiogenesis in rats. These data are in line with results from Goetze et al79 showing a reduction of leptin- and VEGF-induced migration of human ECs by PPARγ agonists. Because neovascularization may contribute to plaque progression as well as aneurysm formation and intraplaque hemorrhage, PPARγ activators might have protective effects in these settings. On the other hand, inhibiting neangiogenesis may have less favorable effects with respect to collateral formation in patients with coronary heart disease, and therefore the definitive role of PPARγ activation in this context remains to be determined. In line with potential atheroprotective effects of PPARγ are studies showing reduction of endothelial intracellular adhesion molecule-1 and VCAM-1 expression by troglitazone or 15d-PGJ2, potentially leading to reduced monocyte/macrophage recruitment and accumulation in a mouse model of atherosclerosis.80,81

VSMCs may also be an important target of PPARγ activators. As such, PPARγ activators inhibit VSMC migration,82 the release of matrix-degrading enzymes,22 and the expression of the angiotensin II type 1 receptor.83 These effects might modulate fatty streak formation and potentially attenuate the arterial response to injury that occurs after coronary intervention. Recent work has implicated PPARγ-dependent pathways in the induction of a differentiated phenotype in proliferative VSMCs. Abe et al84 demonstrated an upregulation of smooth muscle myosin heavy chain and smooth muscle α actin, two specific markers of differentiated VSMCs. This differentiation seems, at least in part, to be mediated by GATA-6–dependent transcriptional mechanisms. Interestingly, Bishop-Bailey et al85 reported increased functional PPARγ in intimal compared with medial VSMCs in rats, potentially reflecting a distinct differentiation status of VSMCs at these locations in the vessel wall. Recent studies highlighted the role of PPARγ in DNA replication and cell-cycle progression in VSMCs. Glitazones were shown to inhibit VSMC growth and proliferation through increased levels of the cyclin-dependent kinase inhibitor p2786 and decreased phosphorylation of the retinoblastoma protein, thus leading to cell-cycle arrest.87,88 In addition, glitazones induce cell apoptosis by increasing the expression of the growth arrest and DNA damage-inducible 45 gene in an Oct-1–dependent manner.89 Pharmacological and genetic approaches demonstrated that PPARγ activation blocks cell-cycle progression and DNA replication by an additional mechanism involving inhibition of platelet-derived growth factor– and insulin-induced minichromosome maintenance protein expression as well as interference with E2F signaling.87

In cells of the monocytic lineage, PPARγ mRNA and protein is present, and its levels increase with differentiation to macrophages.90 Several studies suggest antiinflammatory and potential antiatherogenic effects of PPARγ activators in these cells. The first demonstration of antiinflammatory PPARγ effects in monocyte/macrophages showed that PPARγ agonists inhibit expression of inducible nitric oxide synthase (NOS), scavenger receptor A, and gelatinase B/MMP-9 in monocytes,91 as well as monocyte cytokine expression.71 In addition, PPARγ activators decrease osteopontin expression, enhance the release of the antiinflammatory cytokine IL-1 receptor antagonist, and limit the
expression of myosin heavy chain II markers, suggesting a broad antiinflammatory effect of PPARγ in these cells.92 PPARγ also regulates macrophage lipid homeostasis. On the one hand, PPARγ activators may promote the regression of fatty streaks by increasing the removal of cholesterol from macrophages via the induction of expression of the HDL receptor CLA-1/SRB-1, ABCA1, ABCG1, and apoE.35,93,94 On the other hand, however, PPARγ may also display deleterious effects by increasing glucose metabolism. In vascular cells, the effect of PPARγ on endothelial NO synthase (eNOS) expression, apoptosis, and matrix-degrading enzymes may be regulated via similar mechanisms.

Figure 3. PPARγ modulates insulin-activated signaling pathways in vascular cells. Vascular insulin resistance is characterized by a selective impairment of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway accompanied by an enhancement of the growth factor–stimulated mitogen-activated protein kinase pathway. PPARγ inhibits insulin-induced mitogen-activated protein kinase activation, thus exhibiting a broad spectrum of antiatherogenic effects. In addition, in skeletal muscle, PPARγ enhances insulin-stimulated PI3K and Akt activation, thus affecting glucose metabolism. In vascular cells, the effect of PPARγ on endothelial NO synthase (eNOS) expression, apoptosis, and matrix-degrading enzymes may be regulated via similar mechanisms.

Preclinical/Clinical Data

The effect of PPARγ activators on vascular disease has been examined in various animal models. Law et al.100 were the first to report a reduction of intimal hyperplasia in a rat vascular injury model after troglitazone treatment. The effects of PPARγ activators on lesion development were also examined. Li et al.101 reported that synthetic PPARγ activators reduced lesion size in male LDL receptor–deficient mice. Interestingly, no significant effect was seen in female mice, indicating a sexual dimorphism in response to PPARγ agonists. The decrease in lesion size was associated with decreased expression of TNF-α, reflecting a reduction of the inflammatory response in the vessel wall. In this model, glitazone treatment increased CD36 expression in the vessel wall despite a decrease in overall lesion size. Other groups investigated glitazone effects in apoE-deficient mice and observed similar decreased atherosclerosis in these animals.102,103

Several studies have assessed the effects of glitazones in animal models of ischemia/reperfusion injury and demonstrated their potential benefit in improving contractile function and postischememia heart rate recovery.58,104,105 In addition, glitazones decrease myocardial hypertrophy.106,107 and PPARγ ligands also attenuate myocardial infarction–induced left ventricular dilatation and myocardial hypertrophy and normalize contractile function in vivo in mice.108 Several potential mechanisms may underlie these beneficial actions of glitazones on cardiac function, including antiinflammatory properties on NF-κB/AP-1 as well as decreased production of ET-1 and NO. Thus, PPARγ activation may improve myocardial function, an effect of particular interest for patients with diabetes who are at great risk for myocardial injury.

Although outcome data on the effects of PPARγ activators on cardiovascular mortality are lacking so far, various studies have focused on surrogate markers of atherosclerosis. Studies show that glitazone treatment improves endothelium-dependent vasodilatation but did not affect endothelium-independent vasodilatation,109 suggesting that glitazones act by improving endothelial function. This might be of particular importance in the high-risk population of patients with diabetes, because these patients exhibit early endothelial dysfunction.110 Further evidence of vasoprotective effects of glitazones came from studies with inflammatory surrogate parameters of arteriosclerosis in patients with diabetes, such as C-reactive protein, serum amyloid A, TNF-α, or E-selectin, all known to predict the risk of cardiovascular events in patients.26 Treatment of patients with type 2 diabetes with glitazones significantly reduced C-reactive protein levels as well as white blood cell count and MMP-9 serum levels.111 In addition, a randomized, placebo-controlled trial in patients with coronary artery disease and type 2 diabetes mellitus demonstrated a significant reduction
of serum amyloid A after only 2 weeks of rosiglitazone treatment and a significant decrease in TNF-α levels after 6 weeks, suggesting an effect independent of the metabolic changes induced by the treatment. Patients with type 2 diabetes exhibit elevated levels of other markers, such as sCD40L, MMP-2, MMP-3, and MMP-9, likely reflecting the increased cardiovascular risk of these patients. Glitazone treatment decreased sCD40L levels as well as serum levels of MMP-9 as early as 2 weeks after the initiation of treatment. Interestingly, previous studies have shown that glitazones exhibit maximal glucose-lowering effects only after 8 to 12 weeks.

This difference in the reduction of sCD40L or MMP-9 and glucose strongly suggests that glitazones might directly affect levels of these biomarkers independent of their metabolic action.

In addition to these indirect markers of vascular disease, glitazones influence structural changes in arteriosclerosis and restenosis. In a small clinical study including 135 Japanese patients with diabetes, troglitazone treatment reduced intimal and medial complex thickening in carotid arteries, as determined by B-mode ultrasound. Preliminary data with other glitazones showed similar effects on IMT, suggesting that these structural changes in the vessel wall may be a class effect of these drugs. Given the increased risk of patients with type 2 diabetes to develop restenosis after angioplasty and given the in vitro and in vivo effects of glitazones on smooth muscle cell proliferation and intimal hyperplasia, both critical contributors to restenosis, a Japanese group studied the effect of troglitazone treatment on coronary artery restenosis. After 6 months, compared with placebo, troglitazone significantly reduced narrowing of the coronary lumen. However, at this time, it is not known whether treatment of patients with glitazones will reduce cardiovascular mortality, especially taking into consideration the side effects of these agents, such as fluid retention. Large clinical trials are underway and will demonstrate whether these vasculoprotective effects of glitazones may modulate the clinical outcome in the high-risk population of patients with type 2 diabetes (online Table 1, available at http://circres.ahajournals.org).

**PPARβ/δ and Atherogenesis**

**Cellular Actions**

In vascular cells, most of the work on PPARβ/δ has been performed in cells of the monocytic lineage. Although conflicting data exist, a role for PPARβ/δ in macrophage lipid homeostasis has been demonstrated. On the one hand, PPARβ/δ may promote very-low-density lipoprotein (VLDL) triglyceride and cholesterol loading and storage in macrophages via the induction of scavenger receptors CD36 and SRA as well as ADRP and A/FABP expression. On the other hand, PPARβ/δ activation increases ABCA1 expression and apoA-I-mediated cholesterol efflux. In sharp contrast to these findings, a recent report failed to demonstrate any effect of PPARβ/δ on macrophage cholesterol homeostasis. Besides these effects on macrophage cholesterol homeostasis, a possible role for PPARβ/δ in the control of macrophage inflammation is also emerging. As such, PPARβ/δ activators exert antiinflammatory effects by diminishing LPS-induced inducible NOS and Cox-2 expression.

Although deletion of PPARβ/δ in macrophages results in a lower inflammatory response, suggesting that PPARβ/δ is proinflammatory, incubation of macrophages with a synthetic PPARβ/δ ligand also decreases the production of inflammatory molecules. These apparently paradoxical observations are attributable to an original mechanism of transcription control by PPARβ/δ that depends on whether the receptor is bound to a ligand. In the absence of ligand, PPARβ/δ sequesters a transcriptional repressor of inflammatory response genes, called BCL-6. Therefore, in the absence of PPARβ/δ, BCL-6 actively represses inflammation. However, in the presence of ligand and receptor, a molecular switch occurs and this repressor is released from PPARβ/δ and subsequently exerts antiinflammatory activities. In ECs, the synthetic PPARβ/δ activator L-165041 reduced cytokine-induced VCAM-1 expression as well as the secretion of MCP-1, thus modulating monocyte adhesion to activated ECs. To date, nothing is known about the role of PPARβ/δ in smooth muscle cells and T lymphocytes.

**Preclinical/Clinical Data**

In a mouse model of atherosclerosis, transplantation with bone marrow isolated from PPARβ/δ-deficient mice resulted in reduced atherogenesis, most likely through an increased availability of the inflammatory suppressor BCL-6. To date, the influence of systemic administered PPARβ/δ activators on atherosclerosis has not been reported. Data from clinical studies using PPARβ/δ agonists are presently also lacking.

**Therapeutic Perspectives**

**Selective PPAR Modulators**

Chemically different nuclear receptor ligands can induce distinct agonist or antagonist properties depending on the cell context and the specific target gene. This concept, initially based on observations with estrogen receptor ligands called selective estrogen receptor modulators (SERMs), has been called the selective nuclear receptor modulator concept and adapted to PPARs, the selective PPAR modulator (SPPARM) concept. This model proposes that different PPAR ligands bind to the ligand-binding domain of the PPAR receptor, leading to a different modification of the 3-dimensional conformation of the ligand receptor complex, resulting in differential interaction with cofactors or other transcription factors. Thus, different ligands could activate or repress specific genes depending on the cell type, for example, inducing the favorable PPARγ effects on glucose metabolism without stimulating adipocyte differentiation. The development of SPPARMS is therefore an exciting area of research with potentially large clinical application.

**Combined PPARα/PPARγ Agonists**

Given the favorable metabolic effects of both PPARα and PPARγ activators as well as their potential to modulate vascular disease, combined PPARα/γ activation has recently emerged as an intriguing concept, leading to the development of mixed PPARα/γ activators. Preclinical data in rodents have demonstrated that several of these activators improve insulin sensitivity, as well as FA, glucose, and lipoprotein
metabolism. In addition, clinical data from phase II trials with agents such as ragalitazar confirmed these beneficial effects on insulin sensitivity and HDL and triglyceride levels in patients. However, several patients experienced major side effects, with massive weight gain, appearance of edema, and heart failure, leading to interruption of the development of certain of these agents. Moreover, some of these PPAR activators (online Table 1) will address some of the open questions and prove whether PPAR activation in the cardiovascular system has emerged over the past few years as an intriguing concept to modulate various processes in the development of vascular disease and heart failure. Some data on the overall effect of PPARs in vascular disease remain controversial, but the presently ongoing trials with PPARα and PPARγ activators (online Table 1) will address some of the open questions and prove whether the concept of PPAR activation will translate into improved patient care and potentially broaden the indication spectrum of these PPAR activators.

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**Online Table 1: Ongoing cardiovascular endpoint trials with PPARα and γ agonists**

<table>
<thead>
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<th>Trial</th>
<th>Description</th>
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<tbody>
<tr>
<td>FIELD</td>
<td>Fenofibrate Intervention and Event Lowering in Diabetes</td>
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<tr>
<td>RECORD</td>
<td>Rosiglitazone Evaluated for Cardiac Outcomes and for Regulation of glycaemia in Diabetes</td>
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<tr>
<td>PROACTIVE</td>
<td>PROspective pioglitAzone Clinical Trial In macroVascular Events</td>
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<tr>
<td>DREAM</td>
<td>Diabetes REduction Assessment with ramipril and rosiglitazone Medication</td>
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<td>CHICAGO</td>
<td>A Study Evaluating Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone</td>
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<tr>
<td>PERISCOPE</td>
<td>Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation</td>
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<td>STARR</td>
<td>Study of Atherosclerosis with Ramipril and Rosiglitazone</td>
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<tr>
<td>BARI-2D</td>
<td>Bypass Angioplasty Revascularization Investigation – Type 2 diabetes</td>
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<tr>
<td>RECORD</td>
<td>Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes</td>
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<tr>
<td>ACCORD</td>
<td>Action to Control Cardiovascular Risk in diabetes</td>
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<tr>
<td>ACT NOW</td>
<td>Act Now for the Prevention of Type 2 Diabetes</td>
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