Role of Peroxisome Proliferator-Activated Receptor-γ in Atherosclerosis
– An Update –
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Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the ligand-activated nuclear receptor family. Thiazolidinediones, such as rosiglitazone and pioglitazone, are synthetic agonists selective for PPARγ and have been used in the clinical treatment of type 2 diabetes. However, beyond the metabolic effects on glycemic control, PPARγ and its ligands also have profound effects on cardiovascular biological and pathophysiological processes. As cardiovascular diseases are closely associated with insulin resistance, and the major cause of death and complications of type 2 diabetes, a comprehensive understanding of the cardiovascular roles of this receptor is critical for the rational application of the existing agonists and the future development of therapeutic modulators. Therefore, this review will focus on the recent advances regarding the cardiovascular functions of PPARγ and its recognized effects on major cardiovascular diseases, in particular, atherosclerosis and associated processes. (Circ J 2011; 75: 528–535)

Key Words: Atherosclerosis; Gene expression; Peroxisome proliferator-activated receptor; Myocardial infarction; Thiazolidinediones

Peroxisome proliferators-activated receptors (PPARs) belong to a superfamily of the nuclear hormone receptors that consists of 48 members. PPARs have 3 isoforms, α, β/δ, and γ.1 PPARα is mainly expressed in liver and involved in fatty acid oxidation;2 PPARβ/δ is ubiquitously expressed with a higher level in gut, epidermis, placenta, skeletal muscles and adipose tissue.3 PPARγ is predominantly expressed in adipose tissues and plays a role in adipogenesis and glucose homeostasis.4,5 Differential promoter usage and alternative splicing of the gene generate 4 mRNA isoforms: PPARγ1, γ2, γ3 and γ4. While the latter 2 transcripts encode the same protein as PPARγ1, the PPARγ2 protein has an additional 28 amino acid residues at its N-terminus and is exclusively expressed in adipose tissue.6 PPARs bind to specific DNA responsive elements as heterodimers with the 9-cis retinoic acid receptor RXRα. In the absence of the cognate ligands, PPAR-RXR heterodimers bind a number of co-repressors, including nuclear receptor co-repressor and the silencing mediator of retinoid and thyroid hormone receptor, to suppress the target genes. Upon binding to their selective ligands, PPARs undergo a conformational change that facilitates the dissociation of the co-repressors and the recruitment of co-activators such as steroid receptor co-activator (p160/SRC), CREB-binding protein, p300 and PPARγ co-activator-1α (PGC-1α), leading to the transcriptional activation of the target genes.7–9

Following the first report that eicosanoid 15-deoxy-Δ2,14 prostaglandin J2 (15-D-PGJ2) was an endogenous ligand for PPARγ,10 Number of naturally occurring fatty acid metabolites, including 9- and 13-hydroxyoctadecadienoic acids (9- and 13-HODE), 13- and 15-hydroxyoctadecadienoic acid (13- and 15-HETE) were also found to activate PPARγ.11 Nitro derivatives of unsaturated fatty acids (NO2-FA) are endogenous products of nitric oxide (*NO) and nitrite (NO2−)-modulated reactions that activate PPARγ at nanomolar concentrations.12 It was reported that NO2−–FA act as partial agonists of PPARγ and covalently bind PPARγ.13 Thiazolidinediones (TZDs) or glitazones, including troglitazone (Rezulin, known as Noscal in Japan), rosiglitazone (Avandia) and pioglitazone (Actos) were discovered as selective ligands for PPARγ.14,15 Troglitazone was the first of the TZD class approved in the USA for the treatment of type 2 diabetes mellitus (T2DM) in 1997 and withdrawn 3 years later due to severe hepatotoxicity.16–19 During recent years, wide application of Avandia and Actos as insulin sensitizers has greatly stimulated research in the PPARγ field and rapidly extended our understanding of the biological functions of the receptor and the pharmacological properties of PPARγ agonists. Since PPARγ is also expressed in cardiovascular tissues, such as vascular endothelial cells (ECs),20 smooth
muscle cells (SMCs),\textsuperscript{21} macrophages\textsuperscript{22} and cardiomyocytes, in addition to adipose tissue, and that individuals with T2DM have an increased risk for cardiovascular disease (CVD), the cardiovascular functions of PPAR\(\gamma\) have been under intensive investigation.\textsuperscript{23,24} To date, experiments performed in vitro and in vivo and clinical studies have unequivocally proven that PPAR\(\gamma\) plays an important role in the cardiovascular system and suggest the activation of the receptors may have both favorable and unfavorable effects on the cardiovascular processes. Thus, this review will summarize the most recent progress regarding the role of PPAR\(\gamma\) in CVD, focusing on atherosclerosis and its related pathological processes, including restenosis and aneurysm.

**Atherogenesis**

Atherosclerosis, as a major cause of mortality in T2DM patients, involves many risk factors and complex changes of the vascular components in its progression. These include perturbation/injury of the endothelium, adhesion to and transmigration of monocytes/macrophages into the intima, foam cell formation, and the migration and proliferation of medial SMCs. An initial link between PPAR\(\gamma\) and atherosclerosis was proposed based on the presence of PPAR\(\gamma\) in atherosclerotic plaques\textsuperscript{25} and identification of the components of oxidized low-density lipoprotein (oxLDL) 9- and 13-HODE as its ligands.\textsuperscript{26} Complex effects of PPAR\(\gamma\) were observed, as its activation could inhibit macrophage activation (suppression of iNOS, gelatinase B and scavenger receptor A),\textsuperscript{22} but seemed to promote foam cell formation (uptake of oxLDL via CD36),\textsuperscript{26} raising the possibility that PPAR\(\gamma\) ligands influenced the progression of atherosclerosis.\textsuperscript{27} In vivo studies of different animal models provide evidence supporting a beneficial effect of PPAR\(\gamma\) agonists on atherosclerosis. Li et al demonstrated that rosiglitazone or GW7845 reduced the development of atherosclerosis in male LDL receptor deficiency mice (LDLR\textsuperscript{–/–}).\textsuperscript{28} An atheroprotective effect was also observed for troglitazone in apoE-null mice,\textsuperscript{29} as well as in high fructose- or high fat-fed mice.\textsuperscript{30} The antiatherosclerotic effects of TZDs in these models seemed to be independent of their metabolic effects.\textsuperscript{31,32} A loss-of-function study by transplanting PPAR\(\gamma\)-null bone marrow progenitor cells to LDLR\textsuperscript{–/–} mice showed an increase in atheroma. Bone marrow reconstitution with macrophage-specific knockout of PPAR\(\gamma\) also exacerbated the development of atherosclerosis in LDLR\textsuperscript{–/–} mice, partially due to increased macrophage recruitment and CC chemokine receptor 2 expression.\textsuperscript{34} Recently, Nakay et al reported that pioglitazone treatment in LDLR\textsuperscript{–/–} mice prevented the progression of atherosclerosis from the middle stage of the disease, but was not able to reverse it; pioglitazone treatment had no effects on advanced atherosclerotic lesions.\textsuperscript{35} This was consistent with an earlier finding that pioglitazone had no effect on the atherosclerotic lesion burden in LDLR\textsuperscript{–/–} mice with pre-established lesions.\textsuperscript{36}

**Monocytes/Macrophages**

Monocytes/macrophages play a pivotal role in atherosclerosis. Most of the effects of PPAR\(\gamma\) and its agonists observed in the murine models can be explained by their established roles in macrophage biology. In vitro studies demonstrate that PPAR\(\gamma\) agonists reduce the gene expression and secretion of pro-inflammatory cytokines, including tumor-necrosis factor alpha (TNF\(\alpha\)), interleukin (IL)-1\(\beta\), and IL-6,\textsuperscript{37} and consequently inhibited macrophage activation.\textsuperscript{22} Troglitazone or rosiglitazone modulation of monocyte chemotactic protein 1 (MCP-1) and matrix metalloproteinase-9 (MMP-9) or tissue inhibitor of matrix-metalloproteinase-1 (TIMP-1) production also suggests that PPAR\(\gamma\) may attenuate chemokine-directed transendothelial migration of monocytes.\textsuperscript{38} Thus, together with the suppressed expression of pro-inflammatory molecules on ECs, PPAR\(\gamma\) may reduce atherosclerosis by decreasing the adhesion of monocytes to the vessel walls.

In addition, PPAR\(\gamma\) expressed in macrophage has been shown to affect the atherosclerosis process by regulating LDL uptake and cholesterol efflux.\textsuperscript{39,40} LDL retention and its ensuing oxidation and uptake by infiltrating macrophages lead to the formation of foam cells in the vessel wall. Actually, PPAR\(\gamma\) was first thought to be pro-atherogenic since the agonists increased oxLDL uptake\textsuperscript{25} and, reciprocally, PPAR\(\gamma\) and CD36 were upregulated by oxLDL, thus developing a positive loop to promote foam cell formation.\textsuperscript{39,40} However, subsequent in vivo studies revealed that PPAR\(\gamma\) increased cholesterol efflux from macrophages to inhibit foam cell formation.\textsuperscript{41,42} The reverse trafficking of cholesterol was mediated by ABCA-1, an indirect target gene of PPAR\(\gamma\) via liver X receptor \(\alpha\). Importantly, caveolin-1, another player in cholesterol reverse trafficking, is also a transcriptional target of PPAR\(\gamma\).\textsuperscript{42,43}

**Endothelial Activation**

Endothelial activation is the first step in atherogenesis and characterized by the induced expression of pro-inflammatory adhesion molecules and chemokines such as intercellular adhesion molecules (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin and MCP-1. Synthetic agonists for PPAR\(\gamma\) and 15-D-PGJ\(\beta\) inhibit the pro-inflammatory response in ECs.\textsuperscript{44,45} Constitutive activation of PPAR\(\gamma\) in ECs has a potent antiinflammatory effect.\textsuperscript{46} Suppression of pro-inflammatory gene expression in ECs was demonstrated in the aortae of mice in a TZD-treated atherosclerosis model.\textsuperscript{28} Although activation of PPAR\(\gamma\) is known to inhibit the EC inflammatory response, physiological factors activating this protective system remain less clear. Laminar shear stress, a dragging force acting on the endothelium, has an atheroprotective effect. Shear stress was found to activate PPAR\(\gamma\) via an increase in endogenous ligand(s) to exert its antiinflammatory effects on ECs.\textsuperscript{47} In addition, PPAR\(\gamma\) may also mediate the protective effects of laminar flow on endothelial metabolism,\textsuperscript{50} lipid efflux\textsuperscript{51} and the control of the phenotypic switch of the underlying SMCs.\textsuperscript{52}

**SMC Proliferation and Migration**

SMC proliferation and migration are also critical pathological processes involved in atherosclerosis progression and often induced by multiple stimulating factors, including platelet-derived growth factor, fibroblast growth factor, IL-1, injured ECs and activated platelets.\textsuperscript{53} TZDs have been shown to inhibit the proliferation and migration of both human and rat aortic or coronary vascular SMCs (VSMCs).\textsuperscript{54}–\textsuperscript{59} On the one hand, rosiglitazone and troglitazone promote apoptosis in a PPAR\(\gamma\)-dependent manner via upregulation of GADD45 (growth arrest and transcription of DNA damage-inducible gene 45).\textsuperscript{54} Subsequent study has shown that activated PPAR\(\gamma\) also suppresses telomerase activity and minichromosome maintenance proteins expression in VSMCs.\textsuperscript{58,59} In contrast, SMCs isolated from transgenic mice harboring the dominant-negative mutation in PPAR\(\gamma\) exhibit greater proliferation and migration.\textsuperscript{60} On the other hand, TZDs may also repress SMC proliferation in a receptor-independent pathway via inhibition of ID2, a transcription regulator promoting cell cycle progres-
Pioglitazone suppresses SMC migration induced by homocysteine, a risk factor for coronary heart disease, likely through an inhibition of NAD(P)H oxidase-derived-ROS production and p38 MAPK activation. A most recent study showed that SMC-specific PPARγ deficiency augmented angiotensin II-induced atherosclerosis in LDLR−/− male mice. Pioglitazone inhibited atherosclerosis in wild-type but not the SMC-specific PPARγ deficient mice, pointing to PPARγ in SMCs as a key target for the antiatherosclerotic action of pioglitazone.

**Restenosis**

Restenosis is a major cause of failure of angioplasty. Diabetic patients, particularly, have a significantly higher incidence of restenosis. As in vitro evidence shows that PPARγ strongly inhibits SMC proliferation and migration, a beneficial effect on restenosis was quite conceivable. PPARγ was induced in rat neointima after balloon injury. Studies using animal models were performed to evaluate the PPARγ effect in vivo. Troglitazone, rosiglitazone and pioglitazone all have been reported to inhibit intimal hyperplasia in rodent models of arterial injury. Similarly, local delivery of the PPARγ gene into the carotid artery also reduced neointima formation after balloon injury in rats. In contrast, transgenic mice overexpressing a dominant-negative PPARγ had aggravated intimal hyperplasia. Pioglitazone inhibited in-stent restenosis in hypercholesterolemic rabbits with a reduction of MCP-1 and transforming growth factor-β expression. Finn et al examined the effects of rosiglitazone on re-endothelialization after implantation of sirolimus-eluting, paclitaxel-eluting or bare metal stents in rabbit iliac arteries. They found that re-endothelialization was reduced by rosiglitazone in sirolimus-eluting, but not paclitaxel-eluting or bare metal stents, implying a negative effect on endothelial repair probably due to an inhibition of the mTOR pathway. The overall effects of PPARγ on experimental atherosclerosis and related processes are depicted in the Figure.

In accordance with animal experiments, clinical studies have also shown the efficacy of TZDs in reducing intimal hyperplasia. Marx et al reported that 6-month treatment with pioglitazone significantly reduce neointimal volume and lowered the rate of restenosis after coronary stenting in nondiabetic patients. Koshiyama et al described pioglitazone as decreasing the common carotid arterial intima–media thickness (CIMT) as early as 3 months after administration in 106 Japanese T2DM patients. However, conflicting data have been reported in terms of the effect of TZDs on the risks of restenosis and target revascularization following PCI. Recently, a prospective, multicenter, randomized trial (Prevention of In-Stent Neointimal Proliferation by Pioglitazone Study, POPPS) assessed the efficacy of pioglitazone on instant neointimal suppression in T2DM. After PCI with bare metal stents, T2DM patients were given either pioglitazone or control and assessed by angiography and intravascular ultrasound at 6-month follow-up. The results showed that target
lesion revascularization and the neo-intimal index were significantly lower in the pioglitazone group than in the control group. Angiographic restenosis and the in-stent neo-intimal volume rate were also reduced, although the differences did not reach statistical significance. Clearly, the long-term clinical impact of TZDs on restenosis remains to be addressed.

**Plaque Stability**

Rupture of atherosclerotic plaque leads to thrombosis and acute coronary syndrome (ACS). The morphological features of vulnerable plaques include a thin fibrous cap, large lipid core, increased numbers of inflammatory cells (T lymphocytes, macrophages and mast cells) and a relative scarcity of collagen and SMCs. Increased MMP-9 in both plaque and plasma is a biomarker for carotid plaque instability. High levels of circulating C-reactive protein (CRP) and amyloid A also have predictive value for plaque instability. Zhou et al showed that rosiglitazone modified plaque composition and decreased the number of buried fibrous caps to stabilize vulnerable atherosclerotic plaques in fat-fed ApoE−/− mice. Pioglitazone may also stabilize coronary plaque by reducing the necrotic core component in T2DM patients. The anti-inflammatory effect of PPARγ may account for its plaque-stabilizing effect. Adenovirus-mediated overexpression of PPARγ was reported to stabilize atherosclerotic plaques in ApoE−/− mice, as shown by reduced lipid deposition and macrophages, increased collagen and SMCs in the plaques, accompanied by decreased pro-inflammatory molecules such as MMP-9, tissue factor, MCP-1 etc. In the non-diabetic patient with carotid stenosis, rosiglitazone significantly increases collagen content, decreases the infiltration of inflammatory cells (CD4+ lymphocytes and HLA-DR macrophages) in the plaques and reduces the plasma levels of CRP and amyloid A, suggesting a lesion stabilizing effect. Using a novel dual target molecular imaging technique, Chang et al demonstrated that pioglitazone treatment for 8 weeks reduced MMP and macrophage activity in carotid plaques in ApoE−/− mice receiving a high-cholesterol diet, and provided in vivo evidence indicating the plaque-stabilizing effect of PPARγ. Telmisartan is a unique angiotensin II receptor antagonist with selective PPARγ-activating activity. Compared with treatment using ramipril, an angiotensin-converting enzyme inhibitor, ApoE−/− mice treated with telmisartan showed further reductions in advanced atherosclerotic lesion size and less features of plaque instability such as intraplaque hemorrhage, size of the necrotic core and the number of macrophages. A preliminary study demonstrated that the combination of atorvastatin with pioglitazone could induce a significant regression of coronary atherosclerosis in diabetic and non-diabetic patients, acting on plaque composition or by raising the adiponectin level.

**Aneurysm**

Aneurysm rupture is a common cause of sudden death after myocardial infarction (MI) and stroke. Rosiglitazone reduced aortic expansion and rupture in Ang II-infused ApoE−/− mice along with a suppression of Ang II type A receptor, TNFα, IL-6 and E-selectin. Reduction of lesions in animals pretreated with rosiglitazone is concomitant with decreased expression of inflammatory mediators. Using transplantation of vessels from PPARγ knock-out mice, Hamblin et al demonstrated that loss of PPARγ in vascular SMCs promoted aortic dilatation and elastin degradation and identified cathepsin S as a target gene for PPARγ, suggesting PPARγ as an important contributor in attenuating the development of aortic aneurysms.

**Clinical Trials**

Despite many beneficial effects of PPARγ agonists having been observed in the in vitro and in vivo models of CVD, clinical trials and meta-analyses of clinical studies have been more complicated, with both beneficial and deleterious effects. The prospective pioglitazone clinical trial in macrovascular events (PROactive) examined 5,238 patients with T2DM with preexisting CVD (previous MI, stroke or peripheral vascular disease) randomized to pioglitazone or placebo in addition to other conventional diabetes therapy. In this trial, pioglitazone was found to be associated with a reduction in the combined primary endpoint of a composite of death, nonfatal MI, stroke, major leg amputation, ACS, and coronary or leg revascularization, although the reduction was not significant statistically. However, the principal secondary endpoint (the composite of all-cause death, nonfatal MI and stroke) was significantly reduced by 16%. This study indicated that pioglitazone improved cardiovascular outcome in T2DM patients who have high CVD risk. A substudy of the PRO-active study showed that in 2,445 patients with T2DM and previous MI, pioglitazone reduced the occurrence of fatal and nonfatal MI and ACS. Pioglitazone also reduced recurrent stroke in those with a previous history of stroke. In a subsequent meta-analysis, pioglitazone was associated with a reduced rate of all-cause death and the composite of death, MI and stroke. However, concerns were also raised about the limitations of the PROactive study; specifically, the inclusion of procedure endpoints as primary endpoints as they are less disease-specific, and for a faster closure of the trial. The CHICAGO (Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone) study demonstrated that CIMT, as a surrogate of atherosclerosis, did not progress in T2DM patients treated with pioglitazone whereas those treated with glimepiride showed progression. Similarly, pioglitazone also reduced progression of coronary atheroma volume in a randomized trial (PERISCOPE) performed in patients with T2DM and coronary artery disease, compared with glimepiride. Coronary artery calcium (CAC) is a measure of the total coronary atherosclerotic burden and was analyzed as a secondary endpoint of the CHICAGO study. The result showed that, unlike its beneficial effect on CIMT, pioglitazone did not affect progression of CAC.

Compared to pioglitazone, rosiglitazone has been the target of serious controversy regarding its association with the risk of a cardiovascular outcome. Although the worrisome increase in edema and heart failure has been relatively consistently described for both rosiglitazone and pioglitazone, an increased risk for MI and cardiovascular-related death has been reportedly mainly associated with rosiglitazone. In 2007, a meta-analysis of 42 clinical trials reported a 43% increase of MI and a 64% increase of cardiovascular-related death. However, using alternative meta-analytic approaches that use continuity corrections, Diamond et al showed lower odds ratios that were not statistically significant. They conclude that the risk for MI and death from CVD for diabetic patients taking rosiglitazone is uncertain, with neither increased nor decreased risk established. RECORD (Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes) is a prospective, randomized study including T2DM patients from over 360 centers in 25 countries, the
largest study of a TZD evaluating the associated risk for adjudicated cardiovascular endpoints. This trial did not show that rosiglitazone, when compared with metformin plus sulfonylurea, was associated with an increased risk for MI and total cardiovascular death. However, another meta-analysis of 4 randomized controlled trials found that among patients with impaired glucose tolerance or T2DM, rosiglitazone use for at least 12 months is associated with a significantly increased risk of MI and heart failure but without a significantly increased risk of cardiovascular mortality. Most recently, from a retrospective study, Graham et al report that rosiglitazone was associated with an increased risk of stroke, heart failure and death compared to pioglitazone in T2DM patients aged 65 years and older. Nissen and Wolski analyzed the results of 36 trials containing over 35,000 patients and concluded that rosiglitazone increased the risk for MI, suggesting an unfavorable effect for rosiglitazone.

At the moment, the seemingly different effects of pioglitazone and pioglitazone on cardiovascular outcome are not clear as they are the same class of agonist with similar affinity for the receptor. Suppression of atherosclerosis with pioglitazone therapy was thought to be linked to its ability to raise high-density lipoprotein (HDL) cholesterol. Pioglitazone is associated with significant improvements in triglycerides, HDL cholesterol, LDL particle concentration, and LDL particle size, probably because pioglitazone has a stronger affinity for PPARγ than does rosiglitazone. A recent case-matching cohort study suggests that the risks of MI, heart failure or death are about the same in diabetic patients taking either pioglitazone or rosiglitazone, challenging a number of reports that the cardiovascular risks differ between the 2 drugs.

**Summary**

Rapidly increasing evidence has underscored the importance of PPARγ in CVD, in particular, atherosclerosis. The results from numerous experimental and clinical studies regarding the role of PPARγ in atherosclerosis can be classified into 3 categories: consistent, conflicting, and uncertain. Largely consistent results from in vitro and in vivo animal models suggest that PPARγ and its selective agonists have an atheroprotective role via their effects on inflammatory, proliferative, lipid metabolism or trafficking in macrophages, ECs and SMCs, as well as beneficial effects on endothelial function, whereas the clinical effects of TZDs on heart failure appear to be detrimental. The effects of TZDs on the clinical outcomes of atherosclerosis are conflicting and uncertain. The controversial results can be partially interpreted by differences in the methodological approaches and heterogeneity of the clinical processes. However, the perplexing clinical outcomes indicate our insufficient knowledge of the cardiovascular biology of the nuclear receptors, which is further highlighted when a full agonist is used to treat such complicated clinical scenarios as T2DM. Clearly, in-depth studies are needed to uncover the cardiovascular functions of PPARγ and to safely use its modulators for the treatment of metabolic diseases.

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