Effects of PPARγ on hypertension, atherosclerosis, and chronic kidney disease

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Abstract. Peroxisome proliferator-activated receptor (PPAR) γ is a nuclear hormone receptor that binds to the PPAR response element (PPRE), a direct repeat of AGGTCA spaced by a nucleotide, on DNA as a heterodimer with retinoid X receptor (RXR), and is trans-activated by its ligands including 15-deoxy-Δ12,14-prostaglandin J2 (15dPGJ2) and insulin-sensitizing thiazolidinediones (TZDs) [1] (Fig. 1). Recently, PPARγ has been reported to demonstrate pleiotropic beneficial effects in the vasculatures, independent of its blood glucose-lowering effects. Firstly, PPARγ ligands have been shown to lower blood pressure in both animals and human. The effect may possibly be mediated via the PPARγ-mediated inhibition of the angiotensin (Ang) II type 1 receptor expression as well as Ang II-mediated signaling pathways, which may result in the suppression of the renin-angiotensin system (RAS). Secondly, the progression of atherosclerosis was also prevented by PPARγ ligands in both animals and human. In addition to the PPARγ-mediated suppression of the RAS and the thromboxane A2 system, protective effects of PPARγ ligands on endothelial function may also be involved. Thirdly, reno-protective effects of PPARγ ligands, especially on reducing urinary albumin, have been observed in both animals and human not only in diabetic nephropathy but also in non-diabetic renal diseases. The reno-protective effects may be mediated, at least in part, via the PPARγ ligand-induced blood pressure-lowering effects, protective effects on endothelial function, and vasodilating effects on the glomerular efferent arterioles. Additionally, anti-cancer effects of PPARγ ligands have recently been reported. Taken together, usefulness and effectiveness of PPARγ ligands on lifestyle related diseases will be increasingly appreciated.

Key words: Thiazolidinediones, Angiotensin II, Thromboxane, Endothelium, Kidney

In the present review, we will describe about the recent findings, including our data, regarding the beneficial aspects of PPARγ in the vasculatures.

Effects of PPARγ on Hypertension

Several clinical studies have demonstrated the blood pressure-lowering effect of PPARγ ligand TZDs [3]. Recently, PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study, which included 5,238 enrolled type 2 diabetic patients, have also demonstrated a significant decrease of systolic blood pressure (3 mmHg) by TZD (pioglitazone) treatment [4]. Since the renin-angiotensin system (RAS) plays the most important roles in the progression of hypertension, we examined the effect of PPARγ ligands on angiotensin (Ang) II type 1 receptor (AT1R) expression in vascular smooth muscle cells.
Sugawara et al.

Schematic representation of PPARγ/RXR heterodimer binding to PPRE on DNA.

Fig. 1

Possible mechanisms of PPARγ-mediated transcription suppression of the AT1R gene promoter. Ligand-activated PPARγ may inhibit Sp1 binding to the GC-box-related sequence due to a protein-protein interaction, which may result in the transcription suppression. Co-activator CBP and PPARγ phosphorylation by MAP kinase may modulate the function of PPARγ.

Fig. 2

(VSMCs). Interestingly, 15dPGJ2 as well as TZDs (pioglitazone, troglitazone, and rosiglitazone) dose-dependently decreased the expression of AT1R mRNA [5, 6]. Transcriptional analyses using rat AT1R gene promoter (-1969/+104) and an examination of AT1R mRNA stability using actinomycin D revealed that PPARγ ligands decreased AT1R expression at the gene transcription level, and mutation analyses of the promoter demonstrated that the transcription suppression was mediated via the -58/-34 region (TGC AGA GCA GCG ACG CCC CCT AGG C) of the AT1R gene promoter which contained a GC-box-related sequence (underlined) but no PPREs [5] (Fig. 2). The sequence was demonstrated to be bound to and trans-activated by Sp1 [5]. Transcriptional analyses with over-expression of PPARγ and Sp1, electrophoretic mobility shift assays, and glutathione S-transferase pull-down assays revealed that ligand-activated PPARγ, which could not bind to the GC-box-related sequence, could bind to Sp1 via a protein-protein interaction [5]. Additionally, Sp1 binding to the sequence was inhibited by PPARγ co-incubation [5]. It is therefore speculated that the PPARγ-induced AT1R gene transcription suppression was mediated via the inhibition of Sp1 binding to DNA due to the protein-protein interaction between ligand-activated PPARγ and Sp1 (Fig. 2). Interestingly, the transcription suppression was abrogated by co-activator CERB-binding protein (CBP) over-expression and PPARγ phosphorylation by mitogen-activated protein (MAP) kinase [7], most likely due to the function-
al modification of PPARγ (Fig. 2). The PPARγ ligand-mediated suppression of AT1R expression was also demonstrated in Ang II-infused rats [8, 9]. Moreover, PPARγ ligands have been shown to suppress Ang II-induced phosphatidylinositol 3-kinase and MAP kinase [9], and ameliorate Ang II-mediated inflammatory responses by interfering with the Toll-like receptor 4-dependent signaling pathway [10]. Therefore, PPARγ not only down-regulates AT1R expression, but also inhibits Ang II-mediated signaling pathways, which may result in the suppression of the RAS (Fig. 3). Blood pressure-lowering effects of PPARγ ligands have previously been demonstrated in the Ang II-infused Sprague-Dawley rats [8, 9], spontaneously hypertensive rats [11], deoxycorticosterone acetate-salt rats [12], and hypertensive transgenic mice expressing both human renin and human angiotensinogen transgenes [13]. On the other hand, transgenic mice expressing a dominant negative PPARγ P465L mutation demonstrated hypertension [14], which was consistent to the phenotype of patients who had an equivalent PPARγ P467L mutation [15], without affecting components of the RAS. Taken together, ligand-activated PPARγ may lower blood pressure through several different mechanisms, in addition to the inhibition of the RAS.

**Effects of PPARγ on Atherosclerosis**

Thromboxane (TX) A2 exerts contraction and proliferation of VSMCs, which possibly leads to the progression of atherosclerosis. In order to examine if the TX system was affected by PPARγ, we studied the effects of PPARγ ligands on the expression of TX synthase (TXS) in macrophages [16] and TX receptor (TXR) in VSMCs [6, 17]. Interestingly, PPARγ was observed to suppress both TXS and TXR at the gene transcription level [6, 16, 17]. Detailed analyses revealed that ligand-activated PPARγ inhibited NRF2 (nuclear factor E2-related factor 2) binding to DNA in TXS gene [16], and Sp1 binding to DNA in TXR gene [17], both via protein-protein interactions. Taken together, PPARγ most likely suppress the progression of atherosclerosis through the inhibition of the TX system including both synthesis and action/signal transduction of TXA2 (Fig. 3), in addition to the inhibition of the RAS.

Although atherosclerosis usually proceeded from endothelial dysfunction, PPARγ ligands have been reported to improve endothelial function not only in streptozotocin-induced diabetic rats [18], diabetic db/db mice [19], but also in type 2 diabetic patients [20] and non-diabetic patients with coronary artery disease [21]. Additionally, transgenic mice that specifically
expressed dominant-negative PPARγ in endothelium demonstrated the development of endothelial dysfunction in response to a high-fat diet [22]. PPARγ ligands have also been reported to reduce intimal and medial complex thickening in carotid arteries and in-stent restenosis after coronary intervention in both diabetic and non-diabetic patients [23] as well as neointima formation after balloon injury in rats [24] and in-stent restenosis in atherosclerotic rabbits [25]. In order to examine the direct effects of PPARγ ligands on endothelial gene expression, we performed DNA microarray analyses. Confluent human umbilical vein endothelial cells (HUVEC) were treated with pioglitazone (100 nM), concentration of which was similar to serum concentration of patients after its single oral administration, for 24 hours. Their RNAs were thereafter extracted, and were processed for DNA microarray analyses using Human Genome Oligo Set (Operon Biotechnologies Inc.) representing approximately 35,000 genes. Representative regulated genes are shown in Table 1. Pioglitazone induced expression of tissue inhibitor of metalloproteinases-3, prostacyclin receptor, kallikrein 6 and 11, prostaglandin E2 receptor (EP1 subtype), and microsomal glutathione S-transferase 3, and suppressed expression of matrix metalloproteinase-10 and plasminogen activator inhibitor-2 [26]. PPARγ ligands may therefore be beneficial for the protection of endothelial function in terms of gene expression regulation. Recently, PPARγ ligands have also been reported to stimulate endothelial nitric oxide production in HUVEC [27], and increase number and function of endothelial progenitor cells in patients with coronary artery disease [28]. These observations, in addition to our DNA microarray findings, may contribute to the anti-atherogenic effects of PPARγ ligands.

### Protective Effects of PPARγ in the Kidney

In order to examine intra-renal localization of PPARγ protein, we generated isoform-specific anti-PPARγ antibody and performed immunohistochemical analyses of Sprague-Dawley rat kidneys using the antibody [29, 30]. PPARγ protein was observed to be widely expressed in the nuclei of mesangial and epithelial cells in glomeruli, proximal and distal tubules, the loop of Henle, medullary collecting ducts [29]. Interestingly, PPARγ protein expression was also observed in intima/media of renal vasculatures [29]. We previously reported the vasodilating effects of TZD troglitazone on the glomerular efferent arterioles in the microdissected rabbit kidneys [31]. Considering the immunohistochemical data, the vasodilating effects may possibly be mediated via PPARγ expressed in the intra-renal arterioles. We also observed the induction of PPARγ protein expression in distal tubules and cortical collecting ducts by TZD rosiglitazone administration to Sprague-Dawley rats [30]. These findings are potentially interesting in terms of pathophysiology, since TZDs have been reported to expand body fluid volume through PPARγ stimulation of the epithelial Na+ channel-mediated renal salt absorption [32].

Reno-protective effects of PPARγ ligands on type 2 diabetic patients with nephropathy, especially their effects on reducing urinary albumin, have recently been reported [33]. Additionally, similar effects have been observed in animal experiments using various rodent models of type 2 diabetes [33]. The mechanisms by which PPARγ ligands reduce urinary albumin remain unclear. In addition to their vasodilating effects on the glomerular efferent arterioles [31], their above described effects regarding lowering blood pressure as well as protecting endothelial function may possibly be involved. Additionally, reno-protective effects of PPARγ ligands in non-diabetic renal diseases have recently been reported [34], indicating the general usefulness of PPARγ ligands on chronic kidney disease. We have also demonstrated reno-protective effects of TZD rosiglitazone against cyclosporine-induced renal injury in Sprague-Dawley rats [35].

### Conclusion

More than a decade had passed since the pleiotropic effects of PPARγ was first reported. However, numer-
ous reports regarding novel effects of PPARγ are still publishing every month. In addition to the above described effects against blood pressure, atherosclerosis, and kidney dysfunction, anti-cancer effects of PPARγ ligands have recently been reported [36]. We have also reported the inhibitory effects of PPARγ ligand TZDs on the cell growth of gastrointestinal cancer cell lines [37]. Therefore, usefulness and effectiveness of PPARγ ligands on lifestyle related diseases will be increasingly appreciated.

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