Thiazolidinedione-independent activation of peroxisome proliferator-activated receptor γ is a potential target for diabetic macrovascular complications

Takeshi Matsumura, Kayo Taketa, Seiya Shimoda, Eiichi Araki*

ABSTRACT

Macrovascular complications are responsible for the high morbidity and mortality in patients with diabetes. Peroxisome proliferator-activated receptor γ (PPARγ) plays a central role in the process of adipocyte differentiation and insulin sensitization, and also possesses anti-atherogenic effects. Recently, some statins, angiotensin II type 1 receptor blockers and calcium channel blockers have been reported to activate PPARγ. However, the impact of PPARγ activation on diabetic macrovascular complications is not fully understood. It has been reported that the activation of PPARγ by thiazolidinediones induces anti-atherogenic effects in vascular cells, including monocytes/macrophages, endothelial cells and smooth muscle cells, in atherosclerotic animal models and in clinical studies. We have reported that hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), which are used for treatment of hypercholesterolemia, activate PPARγ and mediate anti-atherogenic effects through PPARγ activation in macrophages. Also, telmisartan, an angiotensin type I receptor blocker, has been reported to have anti-atherogenic effects through PPARγ activation. Furthermore, we have reported that nifedipine, a dihydropyridine calcium channel blocker, can activate PPARγ, thereby mediating anti-atherogenic effects in macrophages. Therefore, statin therapy and part of anti-hypertensive therapy might produce beneficial effects through PPARγ activation in hypercholesterolemic and/or hypertensive patients with diabetes, and PPARγ might be a therapeutic target for diabetic macrovascular complications. In the present review, we focus on the anti-atherogenic effects of PPARγ and suggest potential therapeutic approaches to prevent diabetic macrovascular complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00182.x, 2012)

KEY WORDS: Diabetes, Macroangiopathy, Peroxisome proliferator-activated receptor γ

INTRODUCTION

Diabetic macrovascular complications, including cardiovascular disease (CVD), stroke and peripheral vascular disease, are mainly responsible for the high morbidity and mortality of diabetes patients[1–3]. Several longitudinal epidemiological studies have shown that the risk of mortality from CVD in diabetic subjects is more than double compared with age-matched healthy subjects[4,5], suggesting that some features of diabetes, such as hyperglycemia, must promote an excessive tendency toward CVD.

Hyperglycemia is a diagnostic feature of diabetes, a target for antidiabetic therapy and a marker of glycemic control together with HbA$_{1c}$. Conserving good glycemic control has been associated with a notable reduction in the risk of developing retinopathy, nephropathy and neuropathy in both type 1[6] and type 2[7,8] diabetic patients. Furthermore, when the therapy is started in the early stages of diabetes, intensive diabetes therapy has long-term beneficial effects on the risk of CVD in both type 1and type 2 diabetes[9].

There are several lines of evidence showing that the risk of cardiovascular mortality increases with the elevation in plasma glucose concentrations and HbA$_{1c}$ levels[10,11]. However, although the effect of plasma glucose levels appears to be preponderant in determining the microvascular risk, this does not seem to be the case for macrovascular complications.

The UK Prospective Diabetes Study (UKPDS) clearly shows that for the same degree of HbA$_{1c}$, particularly in its low range, the incidence of myocardial infarction is much greater than that of retinopathy[12]. This apparent paradox can only be resolved by acknowledging the multifactorial nature of the cardiovascular risk in type 2 diabetic subjects. The UKPDS identified several potentially modifiable risk factors, including hypercholesterolemia and hypertension, as well as hyperglycemia, for CVD in

type 2 diabetes[13]. Furthermore, HbA$_{1c}$ was found to be the third most important factor in determining the cardiovascular risk[7]. Indeed, several intervention studies have reported that lipid lowering with hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduces the incidence of cardiovascular events in patients with diabetes[14–20]. In addition, the HOT study[21] and UKPDS 39[22] showed that lowering blood pressure decreased the risk of CVD in hypertensive patients with diabetes. Indeed, several studies have reported that treatment with angiotensin II converting enzyme inhibitor (ACEI)[23], angiotensin II type 1 receptor (AT1R) blocker (ARB)[24] or calcium channel blockers (CCB)[21,25] reduced the incidence of cardiovascular events in hypertensive patients with diabetes. Therefore, it is very important for the prevention of diabetic macroangiopathy to enforce antihypercholesterolemic and antihypertensive therapy in diabetic patients.

Recently, it has been reported that some statins, ARB and CCB activate peroxisome proliferator-activated receptor γ (PPARγ), which plays a central role in the process of adipocyte differentiation, peripheral glucose utilization and insulin sensitization, and has anti-atherogenic effects. In the present review, we examine the possibility of therapeutic approaches for the prevention of diabetic macrovascular complications through PPARγ activation.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ

Peroxisome proliferator-activated receptor γ, a member of the nuclear hormone receptor family of ligand-dependent transcription factors[26], has been well characterized as a regulator of adipogenesis and is abundant in fat cells[27]. Two major splice isoforms of PPARγ, PPARγ1 and PPARγ2, have been identified in mice, whereas in humans and other species at least two other isoforms, PPARγ3 and PPARγ4, have been detected[28].

Several endogenous ligands for PPARγ, including polyunsaturated fatty acids such as linoleic acid, arachidonic acid and eicosapentaenoic acids, have been identified. Prostaglandin-related compounds, such as 15 deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (15d-PGJ$_2$), 9- and 13-hydroxyoctadecadienoic acid, 12- and 15-hydroxyeicosatetaenoic acid and nitro lipids, have also been identified as beneficial PPARγ agonists[29–32]. Thiazolidinediones (TZD), including pioglitazone and rosiglitazone, which are currently on the market, are the most notable synthetic compounds with PPARγ activation properties that improve insulin resistance and lower blood glucose levels in type 2 diabetes.

Peroxisome proliferator-activated receptor γ forms a heterodimer with another nuclear receptor, retinoid X receptor α (RXRα), and binds to a specific DNA sequence, termed PPAR response element 5′-AAGGTCAnAAGGTCA-3′ (PPRE), in the regulatory regions of target genes[33]. In addition, PPARγ can regulate gene expression independently of PPRE, either by suppressing growth hormone protein-1, a transcription factor involved in pituitary-specific gene expression, or by interfering with the function of activator protein (AP)-1, signal transducer and activator

of transcription (STAT)-1 and nuclear factor κB (NF-κB)[34–37]. Coactivators, including steroid receptor coactivator-1, p300/cAMP response element binding protein (CBP), general control non-repressed/p300/CBP-associated factor and PPARγ coactivator, can bind PPARγ/RXRα complexes in a ligand-dependent manner[38]. In contrast, mitotic, stress and inflammatory signals cause PPARγ degradation through phosphorylation on Ser84 of the mouse PPARγ (Ser112 of the human molecule), which is in a consensus mitogen-activated protein kinase (MAPK) target motif PXSPP[39], by extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases and p38 MAPK, which leads to ubiquitination and proteasomal degradation[40]. A Ser84 to Ala84 PPARγ mutant shows increased transcriptional activity, and a similar effect is caused by coexpression with the dual-specificity (Thr/Tyr) MAPK phosphatase, CL100[39].

Peroxisome proliferator-activated receptor γ plays a central role in the process of adipocyte differentiation, peripheral glucose utilization and insulin sensitization in adipocytes[41–44]. In addition, PPARγ is expressed in macrophages[36], endothelial cells (EC)[45], vascular smooth muscle cells (VSMC)[46] and in atherosclerotic lesions[47,48]. PPARγ agonists suppressed the progression of atherosclerosis in low-density lipoprotein (LDL)-receptor-deficient[49,50] or apolipoprotein E-deficient (ApoE$^{-/-}$) mice[51–53] (Table 1). In clinical studies, the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) study suggested that pioglitazone might decrease the occurrence of all-cause mortality, non-fatal myocardial infarction (MI) and non-fatal stroke in patients with type 2 diabetes mellitus and macrovascular diseases[54]. Mazzone *et al.*[55] showed that pioglitazone slowed the progression of carotid intima-media thickness compared with glimepiride in patients with type 2 diabetes. The Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) study showed that treatment with pioglitazone resulted in a significantly lower progression rate of coronary atherosclerosis compared with glimepiride[56]. Ogasawara *et al.*[57] reported that pioglitazone might stabilize coronary plaque by reducing the necrotic-core

Table 1 | Peroxisome proliferator-activated receptor γ ligands suppress the progression of atherosclerosis in animal models

Disease model	Knockout mouse	PPARγ agonist	Decrease in lesion size, %	References
Type 2 diabetes atherosclerosis	LDL-receptor (high-fat diet)	Rosiglitazone	60–80	49
Atherosclerosis	ApoE (normal diet)	Rosiglitazone	18	53
Type 2 diabetes atherosclerosis	LDL-receptor (high-fat diet)	Troglitazone	30	51
Atherosclerosis	ApoE (high-fat diet)	Troglitazone	45	52

ApoE, apolipoprotein E; LDL, low-density lipoprotein; PPARγ, peroxisome proliferator-activated receptor γ.

component in Japanese patients with type 2 diabetes. These findings show the usefulness of PPARγ activator in the treatment of diabetic macroangiopathy. In contrast, meta-analysis reported that treatment with rosiglitazone, but not with pioglitazone, was associated with increased incidence of MI[58,59]. Because meta-analysis has many limitations and the increased risk of MI is still controversial, these reports should be carefully considered. However, several differences between the effects of pioglitazone and rosiglitazone do exist. For example, pioglitazone has more beneficial effects on the lipid profile than rosiglitazone[60]. Furthermore, rosiglitazone, but not pioglitazone, induced cardiac hypertrophy through a PPARγ-independent pathway[61]. To clarify the mechanism(s) of the differences between the effects of pioglitazone and rosiglitazone, further studies are required.

THIAZOLIDINEDIONES

Thiazolidinediones, which are pharmacological ligands of PPARγ, are used to treat patients with type c 2 diabetes. Several anti-atherosclerotic effects of TZD have been reported in various cell types existing in atherosclerotic lesions (Figure 1). In EC, TZD inhibited the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1[62,63], decreased production of chemokines, such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1)[64], decreased endothelin-1, which is involved in the regulation of vascular tone and smooth muscle cell proliferation[45], and increased nitric oxide (NO) release[65]. In addition, a recent report showed that adipose tissue-derived adiponectin plays an obligatory role in TZD-induced improvement of endothelial function in diabetic obese mice[66].

Vascular smooth muscle cells have pivotal roles in the progression of atherosclerosis, and recent studies have shown that they are key targets of TZD. In particular, proliferation of VSMC is one of the critical events in atherosclerosis and vascular intervention-induced restenosis. TZD inhibit these changes in VSMC and neointimal thickening after vascular injury[67]. TZD inhibited VSMC proliferation[67,68] by decreasing phosphorylation of retinoblastoma protein and increasing levels of cyclin-dependent inhibitor p27[67], and thus suppressed neointima thickening after vascular injury[69]. Furthermore, TZD induced apoptosis of VSMC through p53 and Gadd45[70,71]. In addition, TZD inhibited the expression of matrix metalloproteinase-9 (MMP-9)[71] and VSMC migration[46,68], resulting in plaque stabilization. TZD also inhibited IL-1β-induced inducible NO synthase (iNOS) expression and NO production in VSMC[72]. Interestingly, TZD inhibited AT1R in VSMC[73–75], suggesting that this downregulation would be beneficial for diabetic patients with hypertension.

The involvement of PPARγ in regulating lipid metabolism in macrophages was initially suggested by the discovery of the class B scavenger receptor CD36 as a PPARγ target gene in macrophages[76]. CD36, which is involved in the uptake of oxidized LDL (Ox-LDL), is thought to be a critical molecule in
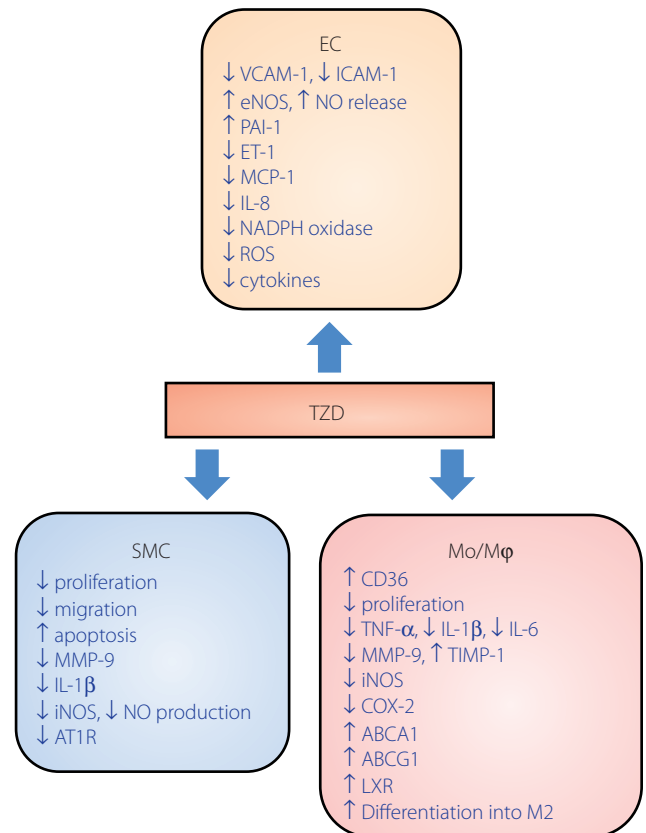

Figure 1 | Anti-atherogenic effects of thiazolidinediones (TZD) in vascular cells. ABCA1, adenosine triphosphate-binding cassette transporter A1; ABCG1, adenosine triphosphate-binding cassette transporter G1; AT1R, angiotensin II type 1 receptor; COX-2, cyclooxygenase-2; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible NO synthase; LXR, liver X receptor; Mφ, macrophage; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase-9; Mo, Monocyte; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SMC, smooth muscle cell; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

macrophage-derived foam cell formation[77]. In addition, CD36 is highly expressed in lipid-laden macrophages in human atherosclerotic plaques[78], and 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid, which are components of the Ox-LDL particle, can activate PPARγ, suggesting that the activation of PPARγ is involved in the formation of foam cells and the progression of atherosclerosis[31]. However, treatment with TZD has been found to increase the apolipoprotein-AI-dependent efflux of cholesterol from both mouse and human macrophages[79,80]. Strikingly, the ability of TZD to promote cholesterol efflux is completely dependent on the expression of adenosine triphosphate-binding cassette transporter A1 (ABCA1), which is characterized as a vital molecule in the control of high-density lipoprotein and apolipoprotein A1-mediated cholesterol efflux

from macrophages[81,82] through PPARγ activation[80]. Thus, although TZD induce the expression of CD36 in macrophages, they simultaneously induce the expression of ABCA1 in these cells, thereby enhancing the removal of cholesterol from the vessel wall through PPARγ activation.

Classically activated macrophages, known as M1 macrophages, express pro-inflammatory cytokines, whereas alternatively activated macrophages, known as M2 macrophages, express anti-inflammatory cytokines and play a protective role in the progression of atherosclerosis. Recent studies showed that PPARγ is one of the key regulators of M1/M2 macrophage polarization[82], and that TZD prime monocytes into M2 macrophages and PPARγ expression is enhanced during differentiation of M2 macrophages[83,84]. TZD suppress the expression of M1 macrophage-associated pro-inflammatory cytokines in monocytes, including tumor necrosis factor-α (TNF-α), IL-1β and IL-6[85], and inhibit macrophage activation *in vitro*[36]. Meanwhile, Marx *et al.*[47] reported that TZD inhibited MMP-9 gelatinolytic activity, an enzyme responsible for plaque rupture. A similar phenomenon was also observed in monocytes[86]. In association with these findings, TZD inhibited MCP-1-directed monocyte migration, which was mediated through a decrease in MMP-9 expression and/or increase in a tissue inhibitor of metalloproteinase-1 expression[87].

One of the characteristic events in atherosclerotic lesions is cell proliferation in arterial walls, including VSMC and macrophages. Indeed, previous *in vivo* studies have reported that macrophages and macrophage-derived foam cells proliferated in atherosclerotic lesions[88–90]. We[91,92] and other groups[93,94] have shown that Ox-LDL enhanced macrophage proliferation and survival *in vitro*. Therefore, it is possible that macrophage proliferation promotes the progression of atherosclerosis. In relation to these findings, we have reported that TZD suppress Ox-LDL-induced macrophage proliferation[95]. Thus, the suppression of macrophage proliferation might be one of the anti-atherogenic effects of TZD.

Peroxisome proliferator-activated receptor γ is thought to alter macrophage function at the molecular level through various mechanisms[38]. PPARγ induces a ligand and a sumoylation-dependent conformational shift that allows direct binding to NF-κB and recruitment of the co-repressor complex, nuclear receptor corepressor, thereby suppressing transcription of NF-κB target genes, such as iNOS[96,97]. TZD also inhibit the activity of other transcription factors, such as AP-1 and STAT-1, both of which are involved in the induction of pro-inflammatory cytokines during differentiation of M1 macrophages[36,85]. As aforementioned, TZD might many anti-atherogenic effects in vascular cells, and might be beneficial for diabetic macroangiopathy beyond the improvement of insulin sensitivity.

CHOLESTEROL-LOWERING THERAPY IN PATIENTS WITH DIABETES

Dyslipidemia, an established risk factor for CVD, is strikingly common in patients with type 2 diabetes, affecting almost 50%

of this population[98]. In addition to hyperglycemia and hypertension, dyslipidemia is a modifiable CVD risk factor that remains largely uncontrolled in patients with diabetes[92]. HMG-CoA reductase inhibitors (statins) are known to reduce the incidence of cardiovascular events and death by functional changes of atherosclerotic lesions[99–102]. Furthermore, because of these benefits, which were shown by many clinical studies, statin therapy is recommended as the initial pharmacological treatment for lowering LDL-cholesterol levels in patients with type 2 diabetes[103,104]. These statin benefits are mainly achieved by its strong lipid-lowering effect. However, recent data suggest that many of the beneficial effects exerted by statins on vascular cells are independent of the cholesterol-lowering effect[105–109]. Cholesterol is synthesized through the isoprenoid biosynthetic pathway[110]. In this pathway, isopentenyl-PP is the basic isoprene unit used for synthesis of all subsequent isoprenoids[110]. Among the isoprenoids, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) serve as important lipid attachments for several proteins, including the small GTP-binding protein Ras and Ras-like proteins, such as Rho, Rac and Cdc42, whose proper membrane localization and function are dependent on isoprenylation[110]. The pleiotropic effects of statins, which include improving the function of EC, VSMC and macrophages[110,111], are thought to be mediated by blocking the synthesis of FPP and GGPP, resulting in the inhibition of small GTP-binding proteins[110].

Several studies reported that atorvastatin and pravastatin increased PPARγ activity in monocytes[112,113]. We also showed that five lipophilic statins (simvastatin, cerivastatin, fluvastatin, atorvastatin and pitavastatin) can activate PPARγ in macrophages[114]. We have shown that statins have a unique mechanism for PPARγ activation (Figure 2). Statin-induced PPARγ activation was suppressed by the addition of mevalonate, FPP or GGPP[114]. Moreover, farnesyl transferase inhibitor and geranylgeranyl transferase inhibitor mimicked the effects of statins. Furthermore, statins inhibited the membrane translocation of Ras, RhoA, Rac and Cdc42, and overexpression of dominant-negative mutants of RhoA and Cdc42, but not of Ras or Rac, increased PPARγ activity[114]. Therefore, suppression of the mevalonate pathway and inactivation of RhoA and Cdc42 are involved in statin-mediated PPARγ activation. In contrast, statins induced ERK1/2 and p38 MAPK activation, and ERK1/2- and p38 MAPK-specific inhibitors abrogated statin-induced PPARγ activation, suggesting that statin-mediated PPARγ activation was caused by the activation of ERK1/2 and p38 MAPK[114]. However, inhibition of RhoA and Cdc42 activated p38 MAPK, but not ERK1/2[114]. Therefore, statin-induced PPARγ activation might be mediated by a RhoA- and Cdc42-dependent p38 MAPK pathway and by a RhoA- and Cdc42-independent ERK1/2 pathway[114]. A subsequent mechanism of PPARγ activation by statins is a cyclooxygenase-2 (COX-2)-dependent increase in 15d-PGJ$_2$, which is a natural ligand of PPARγ[114]. Indeed, we showed that statins increased COX-2 expression and intracellular 15d-PGJ$_2$ levels, and statin-mediated
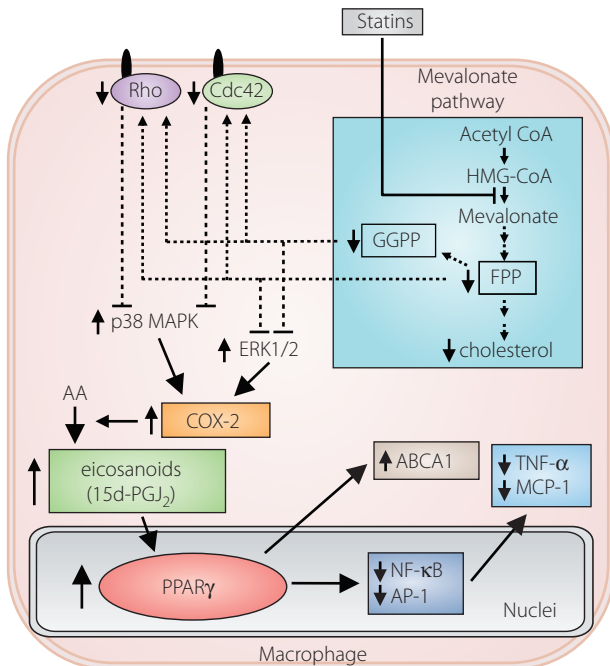
Figure 2 | Summary of statin-mediated peroxisome proliferator-activated receptor γ (PPARγ) activation in macrophages. When treated with statins, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are downregulated by inhibition of the mevalonate pathway, thereby inhibiting geranylgeranylation and farnesylation, and the subsequent translocation of RhoA and Cdc42. The suppression of RhoA and Cdc42 translocation abrogates the RhoA and Cdc42 signaling pathways, thereby inducing p38 mitogen-activated protein kinase (MAPK)-dependent cyclooxygenase-2 (COX-2) production. In addition, small G protein-independent extracellular signal-regulated kinase 1/2 (ERK1/2) activation mediated by the suppression of geranylgeranylation and farnesylation is also involved in COX-2 production. Overexpression of COX-2 produces intracellular 15d-PGJ$_2$, which activates PPARγ. The activation of PPARγ mediates the downregulation of *TNF-α* and *MCP-1* mRNA expression by inactivating AP-1 and NF-κB, and upregulating ABCA1. AA, arachidonic acid; ABCA1, adenosine triphosphate-binding cassette transporter A1; AP-1, activator protein-1; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; 15d-PGJ$_2$, 15-deoxy-Δ^{12,14}-prostaglandin J$_2$; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α.

PPARγ activation was suppressed by selective COX-2 inhibitors or by downregulation of *COX-2* using siRNA[114]. In relation to these findings, it has been reported that statins can induce COX-2 expression in VSMC[115] and macrophages[116].

Several studies have shown that statins suppressed the expression of TNF-α[117] and MCP-1[118] in macrophages. We showed that lipopolysaccharide (LPS)-induced expression of TNF-α and MCP-1 was suppressed by pitavastatin in macrophages, and these effects were abrogated by *PPARγ* siRNA[114]. Furthermore, pitavastatin induced ABCA1 expression, and this effect was also abrogated by *PPARγ* siRNA[114]. Argmann *et al.*[119] also reported that statins induced ABCA1 expression in macrophages, and

this effect was suppressed by the PPARγ antagonist, GW9662. These results suggest that PPARγ activation is one of the key mechanisms in the pleiotropic effects of statins on atherosclerosis, and statin therapy might improve the atherogenic profile by dual effects, such as improving hypercholesterolemia and activating PPARγ, in patients with diabetes.

ANTI-HYPERTENSIVE THERAPY IN PATIENTS WITH DIABETES

Several large randomized trials have reported that ACEI[23], ARB[24] and dihydropyridine CCBs[21,25] prevented cardiovascular events in hypertensive patients with type 2 diabetes. Inhibition of RAS by either ACEI or ARB reduced atherosclerotic lesion formation in experimental studies using various models of atherosclerosis[120–123]. Among the ARB, telmisartan has been identified as a ligand for PPARγ[124,125]. Indeed, we[126] and other groups[127–130] reported that telmisartan suppressed the progression of atherosclerosis in apoE$^{-/-}$ mice (Table 2). Similar results were shown in Watanabe heritable hyperlipidemic rabbits[131] (Table 2). Furthermore, it has been reported that telmisartan prevented ischemic brain damage through PPARγ activation in normal[132] and diabetic mice[133]. Several studies elucidated that telmisartan has direct anti-atherogenic effects on vascular cells (Figure 3). In EC, telmisartan suppresses VCAM-1 expression and reactive oxygen species generation[134], prevents methylglyoxal-mediated cell death[135], increases the permeability of EC[136] and induces proliferation of endothelial progenitor cells[137]. In VSMC, telmisartan suppresses cell proliferation[138] and AT1R expression[139]. We showed that in macrophages, telmisartan inhibits TNF-α and MCP-1 expression through inactivation of NF-κB, induces ABCA1/ABCG1 expression through activation of liver X receptor and inhibits Ox-LDL-induced cell

Table 2 | Telmisartan suppresses the progression of atherosclerosis in animal models

Disease model	Animal models	Decrease in lesion size, %	Dose, mg/kg/day	References
Atherosclerosis	ApoE$^{-/-}$ mice (normal diet)	Male 30	3	[126]
	ApoE$^{-/-}$ mice (normal diet)	Female 32–66 Male 24–57	0.3–3	[127]
	ApoE$^{-/-}$ mice (normal diet)	Male 38	40	[128]
	ApoE$^{-/-}$ mice (high-fat diet)	Male 43	10	[129]
	ApoE$^{-/-}$ mice (high-fat diet)	Male 50	10	[130]
	ApoE$^{-/-}$AT1R$^{-/-}$ mice (high-fat diet)	Male 67	10	[130]
	WHHL rabbit (normal diet)	Male 84	5	[131]

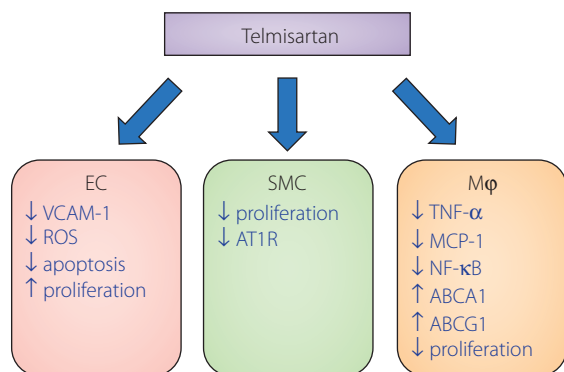ApoE, apolipoprotein E; AT1R, angiotensin II type 1 receptor.

Figure 3 | Anti-atherogenic effects of telmisartan in vascular cells. ABCA1, adenosine triphosphate-binding cassette transporter A1; ABCG1, adenosine triphosphate-binding cassette transporter G1; AT1R, angiotensin II type 1 receptor; EC, endothelial cell; Mφ, macrophage; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; SMC, smooth muscle cell; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.
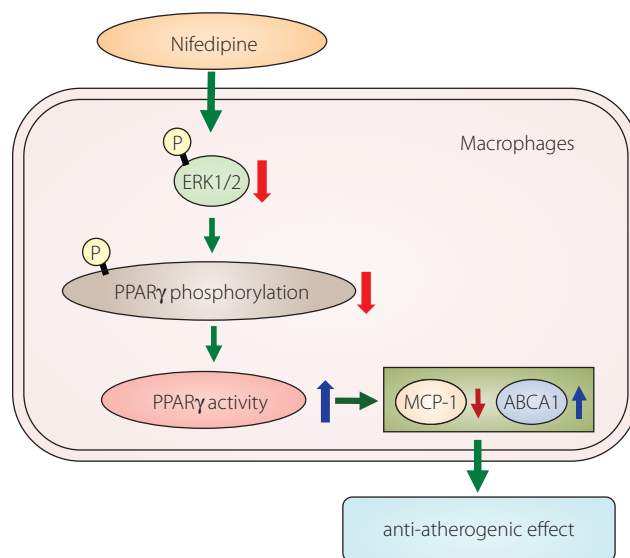

Figure 4 | Summary of nifedipine-mediated peroxisome proliferator-activated receptor γ (PPARγ) activation in macrophages. When macrophages are treated with nifedipine, extracellular-signal regulated kinase 1/2 (ERK1/2) activity is downregulated. Inactivation of ERK1/2 suppresses PPARγ phosphorylation, leading to its activation. PPARγ activation blocks nuclear factor κB (NF-κB) activity, downregulates monocyte chemoattractant protein-1 (MCP-1) expression and upregulates adenosine triphosphate-binding cassette transporter A1 (ABCA1) expression. Nifedipine can therefore induce anti-atherogenic action.

proliferation through activation of PPARγ[126]. Nakaya *et al.*[140] also reported that telmisartan induced ABCA1/ABCG1 expression through PPARγ in THP-1 macrophages. These reports suggest that telmisartan has the capacity to prevent atherosclerosis by not only lowering blood pressure, but also activating PPARγ.

Dihydropyridine CCB are relevant treatments for patients with hypertension. Beyond causing vasodilatation by inhibiting calcium cannels, dihydropyridine CCB provide clinical benefits in patients with coronary artery disease that might be independent of blood pressure reductions[141,142]. Nifedipine, one of the dihydropyridine CCB, reduced the atherosclerotic plaque area in cholesterol-fed rabbits[143–145] (Table 3), and suppressed the progression of atherosclerosis in hypertensive patients[146–150]. Recently, we showed that nifedipine could activate PPARγ in macrophages, which was not observed by amlodipine or Ca^{2+} chelator, suggesting that this effect was not caused by blocking calcium influx[151]. Unlike telmisartan, nifedipine does not act as a direct PPARγ ligand, but suppresses PPARγ phosphorylation

by inhibiting ERK1/2 activity, thereby activating PPARγ (Figure 4)[151]. The exact mechanism of nifedipine-mediated inactivation of ERK1/2 is still unknown. However, Mulvaney *et al.*[152] reported that nifedipine suppressed buserelin (a gonadotropin-releasing hormone agonist)-induced ERK1/2 activation through inactivation of Raf in αT3-1 cells, an immortalized mouse pituitary cell line. In this report, the authors speculated that the inactivation of Raf was caused by blocking L-type calcium channels and, subsequently, inhibiting calcium influx. However, we found that amlodipine did not activate PPARγ[152]. Thus, there might be mechanism(s) other than calcium influx inhibition for nifedipine-mediated inactivation of the Raf/ERK pathway. Hirata *et al.*[153] reported that nifedipine suppressed ERK1/2 activation by inhibiting proline-rich tyrosine kinase-2 (Pyk2) in VSMC. Therefore, inactivation of Pyk2 might also be involved in nifedipine-mediated inactivation of ERK1/2 in macrophages. Further studies are required to clarify the exact mechanism(s) of the nifedipine-mediated inactivation of ERK1/2.

Several studies reported multiple anti-atherogenic effects of nifedipine in vascular cells. Especially in macrophages and human peripheral blood mononuclear cells, nifedipine suppressed the LPS-induced increase of iNOS[154] and inflammatory cytokines, such as TNF-α, IL-1β and interferon-γ[155]. Furthermore, nifedipine inhibited the LPS-induced NF-κB activation[156]. In addition to these previous reports, we recently showed that

Table 3 | Nifedipine suppresses the progression of atherosclerosis in animal models

Disease model	Animal models	Decrease lesion size, %	Dose	References
Atherosclerosis	New Zealand rabbits (high-fat diet)	Male 58	16	143
	WHHL rabbits (high-fat diet)	Male 63	40	144
	Dutch-belted rabbits (high-fat diet)	Male 59	80	145
	ApoE$^{-/-}$ mice (normal diet)	Male 52	10	151

ApoE, apolipoprotein E.

nifedipine suppressed the LPS-induced MCP-1 expression and NF-κB activation through PPARγ activation in macrophages[151]. These findings are supported by a previous report showing that nifedipine suppressed angiotensin II-induced MCP-1 expression through NF-κB inhibition in rat VSMC[157]. In contrast, Suzuki et al.[158] reported that nifedipine induced ABCA1 expression and apolipoprotein-mediated release of cellular cholesterol in macrophages. We also showed that nifedipine induced ABCA1 expression through PPARγ activation[151]. Furthermore, our in vivo study showed that nifedipine suppressed the acceleration of atherosclerosis (Table 3), decreased MCP-1 expression, and increased ABCA1 expression and PPARγ activation in apoE$^{-/-}$ mice[151]. Our recently proposed novel mechanism of nifedipine-mediated PPARγ activation is supported by other reports using the PPARγ antagonist, GW9662[159–161].

Taking these findings into consideration, nifedipine, as well as telmisartan, might have anti-atherogenic effects through PPARγ activation beyond the lowering of blood pressure, and treatment with such anti-hypertensive drugs might be beneficial for the prevention of atherosclerosis in hypertensive patients with diabetes.

CONCLUSIONS

The progression of diabetic macrovascular complications is caused by endothelial injury, activation of macrophages and abnormality of VSMC function. The mechanisms of anti-atherogenic effects by PPARγ are mainly dependent on the improvement of endothelial biology, and inactivation of macrophages and VSMC. Although several studies have shown beneficial effects of TZD on experimental animal models of atherosclerosis, differences in cardiovascular risks among TZD were reported in clinical studies. Further studies are required to clarify the differences among the effects of TZD on diabetic macrovascular complications. Recently, a report showed that not the short-term use, but the use for more than 2 years of pioglitazone was weakly associated with an increased incidence of bladder cancer in a cohort study[162]. In contrast, statins, telmisartan and nifedipine, which possess the ability to activate PPARγ, do not appear to mediate bodyweight gain, edema or incidence of cancers in clinical use. Thus, PPARγ activation mediated by drugs other than TZD, such as statins, nifedipine and telmisartan, might be useful for the prevention of atherosclerosis without severe side-effects. In any case, many in vitro, in vivo and clinical studies have proposed the validity of PPARγ activation in the progression of atherosclerosis. Therefore, a therapeutic approach using PPARγ activation for diabetic macrovascular complications might produce beneficial effects in patients with diabetes.

ACKNOWLEDGEMENTS

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