BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01938.x
www.brjpharmacol.org

REVIEW

Submaximal PPARγ activation and endothelial dysfunction: new perspectives for the management of cardiovascular disorders

Pitchai Balakumar and Sonam Kathuria

Cardiovascular Pharmacology Division, Department of Pharmacology, Institute of Pharmacy, Rajendra Institute of Technology and Sciences (RITS), Sirsa, India

Correspondence
Professor Pitchai Balakumar, Department of Pharmacology, Cardiovascular Pharmacology Division, Institute of Pharmacy, Rajendra Institute of Technology and Sciences (RITS), Sirsa 125 055, India. E-mail: pbala2006@gmail.com

Note: The drug/molecular target nomenclature conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2011).

Keywords
PPARγ partial/submaximal activation; eNOS; EDRF; endothelial function; vascular complications

Received
24 November 2011
Revised
2 February 2012
Accepted
1 March 2012

PPARγ activation plays an important role in glucose metabolism by enhancing insulin sensitization. PPARγ is a primary target for thiazolidinedione-structured insulin sensitizers like pioglitazone and rosiglitazone employed for the treatment of type 2 diabetes mellitus. Additionally, PPARγ activation inhibits adhesion cascades and detrimental vascular inflammatory events. Importantly, activation of PPARγ plays a distinctive role in regulating the physiology and expression of endothelial nitric oxide synthase (eNOS) in the endothelium, resulting in enhanced generation of vascular nitric oxide. The PPARγ activation-mediated vascular anti-inflammatory and direct endothelial functional regulatory actions could, therefore, be beneficial in improving the vascular function in patients with atherosclerosis and hypertension with or without diabetes mellitus. Despite the disappointing cardiac side effect profile of rosiglitazone-like PPARγ full agonists, the therapeutic potential of novel pharmacological agents targeting PPARγ submaximally cannot be ruled out. This review discusses the potential regulatory role of PPARγ on eNOS expression and activation in improving the function of vascular endothelium. We argue that partial/submaximal activation of PPARγ could be a major target for vascular endothelial functional improvement. Interestingly, newly synthesized partial agonists of PPARγ such as balaglitazone, MBX-102, MK-0533, PAR-1622, PAM-1616, KR-62776 and SPPARγM5 are devoid of or have a reduced tendency to cause the adverse effects associated with full agonists of PPARγ. We propose that the vascular protective properties of pharmacological agents, which submaximally activate PPARγ, should be investigated. Moreover, the therapeutic opportunities of agents that submaximally activate PPARγ in preventing vascular endothelial dysfunction (VED) and VED-associated cardiovascular disorders are discussed.

Abbreviations

ADMA, asymmetric dimethyl-L-arginine; AGEs, advanced glycation end products; AMPK, adenosine monophosphate-activated protein kinase; BH4, tetrahydrobiopterin; 15d-PGJ$_2$, 15-deoxy-δ-12,14-PG J$_2$; EDRF, endothelium-derived relaxing factor; eNOS, endothelial NOS; EPCs, endothelial progenitor cells; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; HSP90, heat shock protein90; HUVEC, human umbilical vein endothelial cells; NADPH, nicotinamide adenine dinucleotide phosphate; ox-LDL, oxidized low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; PI3K, phosphotidylinositol 3-kinase; PP1, protein phosphatase 1; RAAS, renin-angiotensin-aldosterone system; SHP-2, protein tyrosine phosphatase-2; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; VCAM-1, vascular cell adhesion molecule-1; VED, vascular endothelial dysfunction

Introduction

PPARγ, a ligand-activated transcription factor of the nuclear hormone receptor family, regulates gene expression for glucose homeostasis. Activation of PPARγ causes insulin sensitization, and thus favours glucose metabolism (Saltiel and Olefsky, 1996; Bishop-Bailey, 2000; Lebovitz and Banerji, 2001). Subsequently, PPARγ agonists were approved for the treatment of insulin resistance-associated type 2 diabetes mellitus (T2DM) (Lebovitz and Banerji, 2001; Dubois *et al.*, 2002). Thiazolidinediones such as ciglitazone, troglitazone, rosiglitazone and pioglitazone are well-studied full agonists of PPARγ, and among them, pioglitazone is the only available PPARγ agonist used clinically to treat T2DM (Forst *et al.*, 2011). The other thiazolidinedione class of drugs are not used clinically due to their adverse profile (Nesto *et al.*, 2003; Balakumar *et al.*, 2007a,b; Quinn *et al.*, 2008; Patel, 2009), and unfortunately even the use of pioglitazone has recently been restricted to a few countries as the US Food and Drug Administration warned that it may cause urinary bladder cancer. Thiazolidinediones, including rosiglitazone, were withdrawn from the market of several countries due to an increased risk of cardiovascular events (Patel, 2009; Palee *et al.*, 2011). Although the adverse profiles of full agonists of PPARγ are highly disappointing, the unexplored therapeutic potential of novel pharmacological interventions targeting PPARγ submaximally (Table 1), for the prevention of cardiovascular disorders, cannot be ruled out.

There is accumulating evidence that activation of PPARγ plays an essential role in the regulation of the vascular endothelial function (Polikandriotis *et al.*, 2005; Duan *et al.*, 2008; Yu *et al.*, 2010). The endothelium is an innermost lining of the blood vessel that is anti-coagulant and anti-thrombotic in nature, thus it maintains the free flow of blood through vessels. It releases various mediators involved in the regulation of vascular tone that include NO, known as

Table 1

Pharmacological agents that submaximally activate PPARγ that need special attention to explore their vascular protective potentials

Serial number	Activators of PPARγ	Endothelial protective potential	References
1	Telmisartan	a) Telmisartan, a partial PPARγ agonist, augmented eNOS expression and activation and NO production through PPARγ activation independently of AT_1 receptor blockade in mice.	Yuen *et al.* (2011)
		b) The diabetes-associated decrease in eNOS-Ser 1177 phosphorylation was normalized by telmisartan in rats.	Wenzel *et al.* (2008)
		c) The protective potential of telmisartan against VED and vascular complications has been predominantly mediated through its PPARγ activating property in obese type 2 diabetic db/db mice.	Toyama *et al.* (2011)
		d) Telmisartan enhanced the bioavailability and vascular generation of NO through its PPARγ-agonistic action in WHHL rabbits.	Ikejima *et al.* (2008)
		e) Telmisartan improved VED by reducing ADMA levels through its additional mechanism associated with PPARγ activation independent to its AT_1 receptor blockade-mediated hypotensive action in patients with essential hypertension.	Ono *et al.* (2009)
2	Rosuvastatin	Rosuvastatin regulated blood pressure homeostasis via PPARγ-NO-dependent mechanism in obese dyslipidaemic mice.	Desjardins *et al.* (2008)
3	Atorvastatin	The anti-atherogenic effect of atorvastatin has been associated with an increase in the expression of PPARγ, enhancement of NO concentration and decrease in PAI-1 level in rabbits.	Yang *et al.* (2010)
4	Balaglitazone	It is a partial PPARγ agonist having reduced potential to increase fluid retention and weight gain. Its endothelial protective role is yet to be determined.	Henriksen *et al.* (2011)
5	MBX-102	It is a partial PPARγ agonist not having classical PPARγ agonists-associated side effects. Its endothelial protective role is yet to be determined.	Gregoire *et al.* (2009)
6	MK-0533	It is a partial PPARγ agonist having reduced potential to increase plasma and extracellular fluid volume. Its endothelial protective role is yet to be determined.	Acton *et al.* (2009)
7	PAR-1622	It is a selective partial PPARγ agonist having excellent anti-hyperglycemic activity with broad safety profile against fluid retention experimentally. Its endothelial protective role is yet to be determined.	Kim *et al.* (2009a)
8	PAM-1616	It is a selective partial PPARγ agonist having excellent anti-hyperglycemic activity with reduced tendency to cause fluid retention experimentally. Its endothelial protective role is yet to be determined.	Kim *et al.* (2011)
9	KR-62776	It is a partial PPARγ agonist with reduced tendency to cause obesity experimentally. Its endothelial protective role is yet to be determined.	Kim *et al.* (2009b)
10	SPPARγM5	It is a potent partial PPARγ agonist not causing significant fluid retention or cardiac hypertrophy in obese Zucker rats. Its endothelial protective role is yet to be determined.	Chang *et al.* (2008)

endothelium-derived relaxing factor. NO is a key regulator of cardiovascular function as it mediates vasorelaxation, inhibits leucocyte–endothelial adhesion and prevents platelet aggregation (Naseem, 2005; Desjardins and Balligand, 2006; Balakumar *et al.*, 2008a; Jindal *et al.*, 2008; Kaur *et al.*, 2010a), the actions of which could be of benefit in averting the pathogenesis of cardiovascular disorders such as atherosclerosis, hypertension and ischaemic heart disease.

NO is synthesized in the endothelium from L-arginine by endothelial NOS (eNOS) (Palmer *et al.*, 1988; Wyatt *et al.*, 2004). Vascular endothelial dysfunction (VED) is specified as an impairment of endothelium-dependent vasorelaxation resulting from eNOS down-regulation or inactivation, and the subsequent reduction in NO levels, leading to deregulation of vascular homeostasis and induction of cardiovascular dysfunction (Rush *et al.*, 2005; Balakumar *et al.*, 2008a,b; Balakumar and Kaur, 2009). In addition, high oxidative stress may account for the development of VED as the superoxide anion radical ($O_2^{\cdot-}$) combines with NO to decrease the bioavailability of endothelial NO. Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase is known to produce superoxide anion, while superoxide dismutase (SOD) degrades superoxide anion. The overexpression of NADPH oxidase and reduced expression of SOD could therefore cause increased generation of superoxide anion, which could reduce the bioavailability of endothelial NO (Hwang *et al.*, 2005), leading to the development of a dysfunctional endothelium. The VED has been associated with various cardiovascular disorders such as atherosclerosis (Desjardins and Balligand, 2006), hypertension (Puddu *et al.*, 2000) and coronary artery disease (Caramori and Zago, 2000). Therefore, maintaining a normal function of the vascular endothelium by maintaining the normal activation of eNOS and generation of NO in the vascular bed is essential for the prevention of the progression of detrimental cardiovascular disorders. Interestingly, recent studies have demonstrated a key role of PPARγ in regulating the function of the vascular endothelium (Ríos-Vázquez *et al.*, 2006; Duan *et al.*, 2008; Kaur *et al.*, 2010b; Yu *et al.*, 2010). There is evidence that activation of PPARγ causes eNOS activation and NO generation involving diverse mechanisms (Calnek *et al.*, 2003; Cho *et al.*, 2004; Polikandriotis *et al.*, 2005). It is important to identify the signalling system involved in PPARγ-mediated eNOS activation for advancing our current knowledge of the beneficial role of novel PPARγ agonists in preventing the induction and progression of VED. In this review we discuss the potential regulatory role of PPARγ on eNOS expression and activation, and novel therapeutic openings of submaximal (diminished maximal) PPARγ agonists in preventing VED and VED-associated cardiovascular disorders.

Mechanism of eNOS activation

The activation of eNOS for the generation of endothelial NO is regulated by various kinases and phosphatases. Structurally, eNOS consists of two terminal domains, oxygenase-NH_2 and reductase-COOH, and it also has a few well-described sites for phosphorylation/dephosphorylation such as Ser^{1177} and Thr^{495}. Some other sites that regulate eNOS activity, Ser^{633}, Ser^{114} and Ser^{615}, have also been identified; however, their precise roles remain controversial (Mount *et al.*, 2007). The eNOS has a binding site for calmodulin necessary for the enzyme activity (Mount *et al.*, 2007). The regulation of eNOS expression and activation is influenced by various cellular events such as transcriptional regulation, protein–protein interaction, phosphorylation and dephosphorylation at different amino acid sequences of eNOS (Govers and Rabelink, 2001).

The eNOS is chiefly expressed in the vascular endothelium. Shear stress, induced by flow of fluid across the endothelium, can up-regulate the expression of eNOS (Noris *et al.*, 1995). In addition, various growth factors such as vascular endothelial growth factor, basic fibroblast growth factor and epidermal growth factor up-regulate the expression of eNOS (Kroll and Waltenberger, 1998; Zheng *et al.*, 1999). Moreover, insulin has been shown to play a key role in the up-regulation of eNOS (Kuboki *et al.*, 2000). Interestingly, a low concentration of oxidized low-density lipoprotein (ox-LDL) has been shown to up-regulate eNOS (Hirata *et al.*, 1995). Conversely, a high concentration of ox-LDL down-regulates the expression of eNOS (Laufs *et al.*, 1998). It should be noted that excessive NO itself can reduce eNOS expression through cGMP-mediated activation of a negative feedback regulatory mechanism (Vaziri and Wang, 1999).

The binding of eNOS to caveolin-1 in endothelial cells results in eNOS inactivation (Ju *et al.*, 1997). The protein–protein interaction between eNOS and caveolin-1 markedly reduces eNOS activity because caveolin-1 hampers calmodulin binding to eNOS when cytosolic calcium levels are low (Michel *et al.*, 1997). On the other hand, the interaction of heat shock protein 90 (HSP90) with eNOS results in eNOS activation (Garcia-Cardena *et al.*, 1998). Interestingly, protein–protein interaction between HSP90 and eNOS enhances eNOS activity by inducing calmodulin-stimulated displacement of eNOS from caveolin-1 (Gratton *et al.*, 2000). In addition, HSP90 and eNOS interact to enhance PKB (Akt)-mediated eNOS activation in the endothelium (Fontana *et al.*, 2002; Takahashi and Mendelsohn, 2003).

As summarized in Table 2, the eNOS activity is determined through phosphorylation or dephosphorylation at Ser^{1177}, Ser^{633} and Thr^{495} sites of eNOS by multiple protein kinases, including PKB, PKA, PKC, AMP-activated protein kinase (AMPK) and ERK, and phosphatases such as protein phosphatase (PP)1 and PP2A in response to multiple stimuli via shear stress, growth factors, insulin, etc. (Dimmeler *et al.*, 1999; Michell *et al.*, 1999; 2001; Fleming *et al.*, 2001; Mount *et al.*, 2007; Chen *et al.*, 2009; Xiao *et al.*, 2011). Dimmeler *et al.* (1999) showed that PKB phosphorylates eNOS at the Ser^{1177} site by a Ca^{2+}-independent regulatory mechanism to activate eNOS. The PKA signalling activates eNOS by enhancing the phosphorylation of Ser^{1177} and dephosphorylation of Thr^{495}, whereas the PKC signalling in endothelial cells inhibits eNOS activation by dephosphorylating Ser^{1177} and phosphorylating Thr^{495} (Michell *et al.*, 2001). AMPK has been shown to phosphorylate Thr^{495} *in vitro* (Chen *et al.*, 1999); however, the same has not been demonstrated *in vivo*, indicating a conflicting role for AMPK in the regulation of eNOS activity. However, Chen *et al.* (2009) recently demonstrated that Ser^{633} phosphorylation could be important for endothelial NO production, and AMPK phosphorylates eNOS at Ser^{633} in endothelial cells to generate NO

Table 2

Regulation of eNOS action by multiple protein kinases and phosphatases

Serial number	Kinases and phosphatases	eNOS phosphorylation site	Status of eNOS	References
1	PKB	Ser^{1177} phosphorylation	Activation	Dimmeler *et al.* (1999)
2	PKA	Ser^{1177} phosphorylation and Thr^{495} dephosphorylation	Activation	Michell *et al.* (2001)
3	PKC	Ser^{1177} dephosphorylation and Thr^{495} phosphorylation	Inactivation	Michell *et al.* (2001)
4	AMPK	Ser^{633} phosphorylation	Activation	Chen *et al.* (2009)
5	ERK1/2	Ser^{633} phosphorylation	Activation	Xiao *et al.* (2011)
6	PP1	Thr^{495} dephosphorylation	Inactivation	Fleming *et al.* (2001); Michell *et al.* (2001)
7	PP2A	Ser^{1177} dephosphorylation	Inactivation	Michell *et al.* (2001); Mount *et al.* (2007)

(Chen *et al.*, 2009). Xiao *et al.* (2011) reported that ERK1/2 activation activates eNOS by enhancing Ser^{633} phosphorylation in response to endoplasmic reticulum Ca^{2+} release. Among the phosphatases, PP1 could dephosphorylate Thr^{495} to activate eNOS, while PP2A could dephosphorylate Ser^{1177} to inactivate eNOS (Fleming *et al.*, 2001; Michell *et al.*, 2001; Mount *et al.*, 2007). Taken together these results indicate that upon activation in response to signalling stimuli, eNOS generates NO from L-arginine, one of the most common endogenous amino acids, in the presence of molecular oxygen and NADPH as substrates, and tetrahydrobiopterin (BH4), flavin adenine dinucleotide, flavin mononucleotide as cofactors (Palmer *et al.*, 1988; Govers and Rabelink, 2001).

The regulatory role of PPARγ in eNOS expression and activation and NO generation in conjunction with therapeutic potentials of PPARγ ligands in improving the function of vascular endothelium

PPARγ is mainly expressed in white and brown adipose tissue and also in endothelial cells and vascular smooth muscle cells (Tontonoz *et al.*, 1995; Kota *et al.*, 2005). As mentioned in the previous section, PPARγ agonists are used to specifically augment insulin sensitivity and to counter insulin resistance in T2DM patients. It is a clear that pharmacological agents that up-regulate and activate eNOS and enhance the generation and bioavailability of NO could be of therapeutic value in preventing the induction and progression of cardiovascular disorders, including atherosclerosis, hypertension and ischaemic heart disease. Recent studies have suggested a potential regulatory role of PPARγ on eNOS expression and activation and NO generation in the vascular endothelium. The following section addresses this imperative issue.

Administration of PPARγ activators such as rosiglitazone and pioglitazone in angiotensin-II-infused rats prevented the development of hypertension, reversed vascular remodelling, reduced vascular inflammation and improved endothelial function (Diep *et al.*, 2004). Activation of PPARγ using 15-deoxy-δ-12,14-PGJ_2 (15d-PGJ_2) or ciglitazone was shown to stimulate the release of NO from the endothelium to protect the vascular wall (Calnek *et al.*, 2003). Interestingly, this study demonstrated that the PPARγ-mediated release of NO might be independent of eNOS expression as both 15d-PGJ_2 and ciglitazone did not alter eNOS mRNA levels. It was suggested that a direct transcriptional mechanism could have been involved in PPARγ-mediated release of NO in endothelial cells (Calnek *et al.*, 2003). However, Polikandriotis *et al.* (2005) suggested that PPARγ activation could indirectly activate eNOS through a HSP90-dependent mechanism. The authors investigated the molecular mechanism underlying PPARγ activation-mediated increase in endothelial NO production. Treatment of human umbilical vein endothelial cells (HUVEC) with PPARγ agonists such as 15d-PGJ_2, ciglitazone or rosiglitazone for 24 h was found to increase NO release. However, co-administration of GW9662, a selective PPARγ antagonist, inhibited the increase in NO release induced by 15d-PGJ_2, ciglitazone or rosiglitazone implicating a key role for PPARγ in the induction of endothelial NO release (Polikandriotis *et al.*, 2005). Interestingly, rosiglitazone and 15d-PGJ_2, but not ciglitazone, stimulated HSP90–eNOS interaction, followed by eNOS activation (at Ser^{1177} phosphorylation). This suggests that PPARγ ligands have differential effects on eNOS-mediated release of NO from the endothelium. Moreover, in order to confirm the intermediate role of HSP90 in PPARγ activation-mediated eNOS activation and NO generation, the authors of this study investigated the effect of co-administration of the HSP90 inhibitor, geldanamycin; this was noted to attenuate 15d-PGJ_2- and rosiglitazone-stimulated eNOS activation and NO release from endothelial cells, confirming the key role of HSP90 in this context (Polikandriotis *et al.*, 2005).

The elevated vascular oxidative stress is known to reduce endothelial bioavailability of NO. The oxygen free radicals combine with NO to form peroxynitrite, resulting in the reduced bioavailability of NO (Ferdinandy and Schulz, 2003). Hwang *et al.* (2005) investigated the effect of PPARγ ligands on superoxide anion generation-induced NO metabolism. Treatment with 15d-PGJ_2 or ciglitazone for 24 h decreased HUVEC membrane NADPH-dependent superoxide anion

generation by reducing relative mRNA levels of the NADPH oxidase subunits such as nox-1, gp91phox (nox-2) and nox-4, which was accompanied by an enhanced expression of SOD. The authors suggested that, in addition to stimulating NO release from the endothelium, PPARγ activation could also enhance NO bioavailability by reducing endothelial superoxide anion generation and oxidative stress (Hwang *et al.*, 2005). This study further revealed the underlying molecular mechanism involved in PPARγ-mediated regulation of NO physiology. Recently, it has been shown that oxidative stress attenuates PPARγ expression and activity in vascular endothelial cells through activation of inhibitory redox-regulated transcription factors and suppression of PPARγ transcription (Blanquicett *et al.*, 2010). Thus, PPARγ agonists, through a reduction in oxidative stress as reported by Hwang *et al.*, (2005), could activate their own PPARγ-mediated transcriptional programme for the regulation of the function of vascular endothelium.

Yuen *et al.* (2011) have recently suggested that PPARγ activation up-regulates eNOS expression. In this study, telmisartan, an AT_1 receptor blocker having PPARγ agonistic property, inhibited vasoconstriction in mice resistance arteries that was noted to be mediated through a PPARγ-dependent increase in eNOS expression and activation, independent of its classical AT_1 receptor blocking ability (Yuen *et al.*, 2011). Likewise, Toyama *et al.* (2011) suggested a direct role of PPARγ in providing vascular protection in the obese type 2 diabetic db/db mouse. In this study, telmisartan was noted to significantly ameliorate VED and the reduction in eNOS phosphorylation/activation in diabetic db/db mice. However, co-administration of GW9662 abolished these protective effects of telmisartan against VED in diabetic db/db mice (Toyama *et al.*, 2011), confirming the regulatory role of PPARγ in eNOS activation in providing vascular protection. In addition, treatment of Dahl salt-sensitive hypertensive rats with telmisartan effectively inhibited the vascular lesion formation such as medial thickness and perivascular fibrosis, but telmisartan plus GW9662 had no inhibitory effects, suggesting that the vasculoprotective effect of telmisartan is mediated through activation of PPARγ (Kobayashi *et al.*, 2008). Moreover, telmisartan was noted to decrease plasma levels of asymmetric dimethyl-L-arginine (ADMA), an endogenous inhibitor of eNOS, and subsequently improve vascular function in patients with essential hypertension; an effect that might be mediated via its PPARγ activating property (Ono *et al.*, 2009). Furthermore, Li *et al.* (2010) reported that the young male spontaneously hypertensive rat (SHR) showed significantly reduced expression of phosphotidylinositol 3-kinase (PI3K) and decreased phosphorylation of PKB-eNOS in vascular tissues. However, treatment with rosiglitazone increased vascular PPARγ expression, which was noted to be accompanied by restoration of PI3K/PKB/eNOS signalling activation, followed by improvement of endothelial function in the young SHR (Li *et al.*, 2010).

Diabetes mellitus-induced VED is associated with elevated levels of advanced glycation end products (AGEs). Liang *et al.* (2009) showed that AGEs could cause apoptosis of endothelial progenitor cells (EPCs), resulting in dysfunction of these cells. These authors investigated the role of PPARγ activation in AGEs-induced dysfunction of EPCs. Interestingly, PPARγ activation by rosiglitazone reduced the apoptosis of EPCs and attenuated the dysfunction of EPCs induced by AGEs via up-regulation of PKB and eNOS signalling of EPCs (Liang *et al.*, 2009). Likewise, PPARγ activation using pioglitazone up-regulated PKB and eNOS phosphorylation resulting in the amelioration of VED and enhancement of blood flow recovery after tissue ischaemia in the diabetic mouse (Huang *et al.*, 2008).

The mechanism involved in PPARγ activation-mediated eNOS activation and improvement of endothelial function is not completely understood, although the key role of HSP90 was suggested by Polikandriotis *et al.* (2005) in this context. Additionally, Wong *et al.* (2011) have recently suggested that adiponectin, a hormonal protein secreted from adipose tissue, could play a key role in PPARγ activation-mediated eNOS activation and subsequent improvement of endothelial function. The authors demonstrated that PPARγ activation by rosiglitazone in the diabetic db/db mouse stimulated the release of adiponectin, which further activated AMPK/eNOS and cAMP/PKA signalling pathways in the aorta, resulting in a reduction in oxidative stress and the enhancement of NO bioavailability, leading to an improvement in endothelial function (Wong *et al.*, 2011). Intriguingly, the authors observed that PPARγ activation restored the aortic endothelium-dependent relaxation in the diabetic mouse, whereas the diabetic mouse lacking adiponectin did not respond (Wong *et al.*, 2011), suggesting strongly that adiponectin could mediate PPARγ-induced eNOS activation and NO generation to improve the function of the vascular endothelium.

It is well known that Rho-kinase, a serine–threonine kinase, plays a pivotal role in inducing VED by inactivating eNOS and reducing NO generation (Budzyn *et al.*, 2006; Nohria *et al.*, 2006). Intriguingly, Wakino *et al.* (2004) reported that activation of PPARγ inhibited Rho-kinase by up-regulating protein tyrosine phosphatase-2 (SHP-2). It should be noted that Vav, a GTP/GDP exchange factor, activates Rho-kinase. Thus Vav could be dephosphorylated by SHP-2. With this in mind, Wakino *et al.* (2004) convincingly demonstrated that PPARγ activation by pioglitazone in angiotensin-II-treated rat cultured aortic smooth muscle cells up-regulated SHP-2, which subsequently dephosphorylated Vav, resulting in inactivation of Rho-kinase. Importantly, Wakino *et al.* (2004) demonstrated that a similar mechanism was associated with PPARγ-mediated inhibition of Rho-kinase through up-regulation of SHP-2 in aortic tissues isolated from the SHR (Wakino *et al.*, 2004). Taken together these findings indicate that PPARγ-mediated up-regulation and activation of eNOS could involve HSP90, adiponectin and a Rho-kinase associated signalling mechanism (Figure 1).

Is submaximal activation of PPARγ a valuable approach in the prevention of VED and VED-associated cardiovascular disorders?

The full agonists of PPARγ have been associated with severe adverse events. Although the adverse profiles of PPARγ agonists are highly disappointing, we cannot rule out the unexplored therapeutic potentials of pharmacological agents that

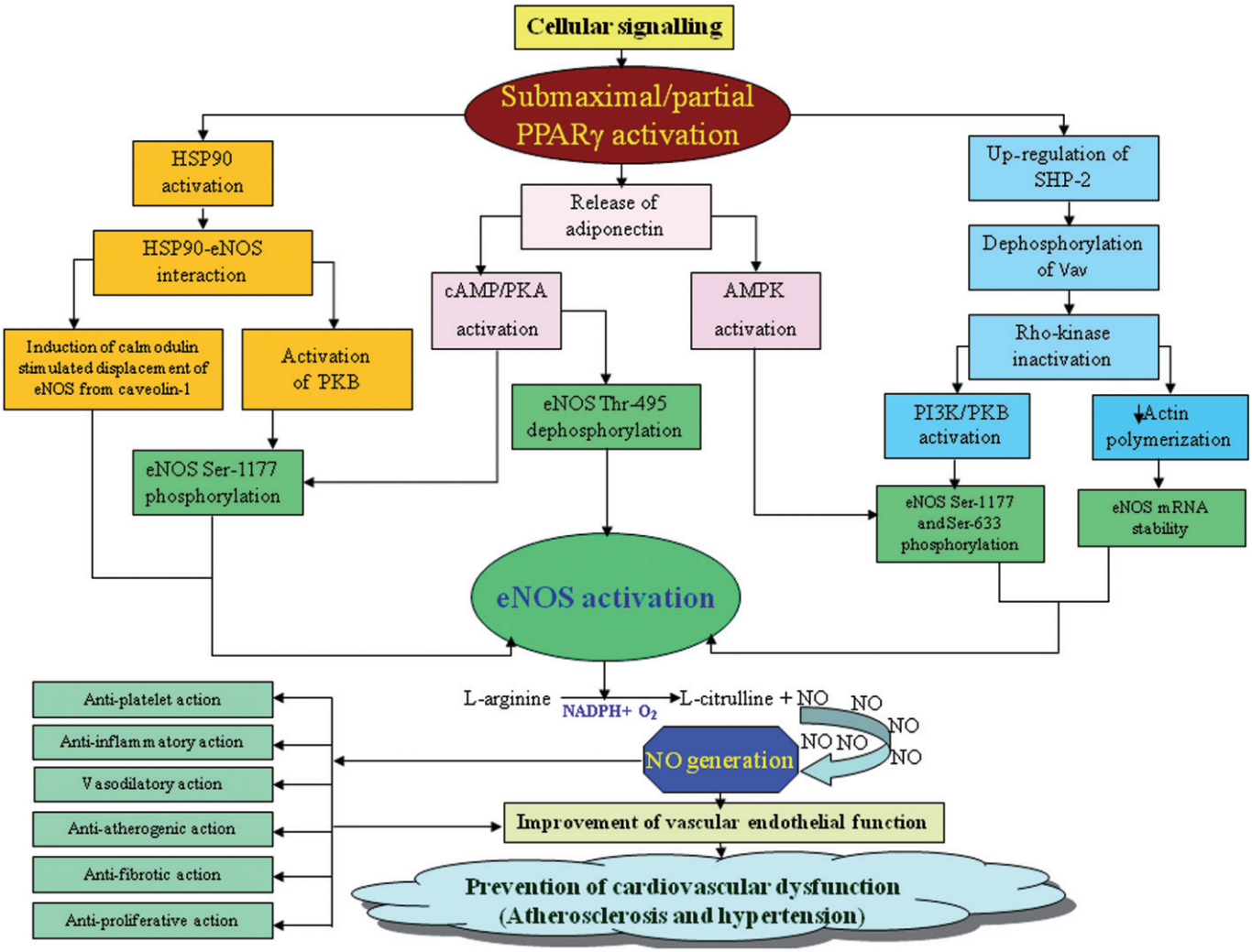

Figure 1

The cellular signalling system regulating PPARγ-mediated eNOS activity through phosphorylation/dephosphorylation at various sites of eNOS.

differentially target PPARγ in improving cardiovascular abnormalities. It is a matter of debate as to what extent PPARγ needs to be activated to achieve a desirable insulin sensitizing effect and consequently avoid major adverse events. In fact, full agonists of PPARγ such as ciglitazone and troglitazone have already been withdrawn from the market, and the clinical use of rosiglitazone and pioglitazone for the treatment of T2DM is under question due to their adverse profiles. Rosiglitazone, a well-studied PPARγ agonist, has been withdrawn from the market in several countries due to an increased risk of cardiovascular events (Palee *et al.*, 2011). We strongly believe that activating PPARγ at its maximal level may perhaps have undesirable effects on cardiovascular system, while submaximal/partial activation of PPARγ may have beneficial cardiovascular effects as seen with the telmisartan, a partial activator of PPARγ. Moreover, it is worth mentioning that the newly synthesized PPARγ partial agonists (Table 1 and Figure 2) such as balaglitazone, MBX-102, MK-0533, PAR-1622, PAM-1616, KR-62776 and SPPARγM5 have been reported to be devoid of or have a reduced tendency to cause the adverse effects associated with full agonist of PPARγ (Chang *et al.*, 2008; Acton *et al.*, 2009; Gregoire *et al.*, 2009; Kim *et al.*, 2009a,b; 2011; Henriksen *et al.*, 2011). We, therefore, propose that pharmacological agents that submaximally activate PPARγ need to be further investigated for their possible cardiovascular protective potential. This contention has been convincingly delineated in the following section.

Telmisartan is a safe and long-acting AT_1 receptor blocker effectively used as monotherapy for treating essential hypertension and hypertension-associated cardiovascular and renal disorders (Niegowska *et al.*, 2005; Ruilope, 2011). The cardiovascular and renal functional improvements associated with telmisartan are not only mediated through its AT_1 receptor blocking property but also through its partial PPARγ agonistic property (Benson *et al.*, 2004; Schupp *et al.*, 2004; Yamagishi and Takeuchi, 2005). It is worth noting that telmisartan affords cardiovascular protection exclusively through its ability to act as a partial PPARγ agonist, thereby activating eNOS and improving the endothelial function, independently of its AT_1 receptor block properties (Kobayashi *et al.*,

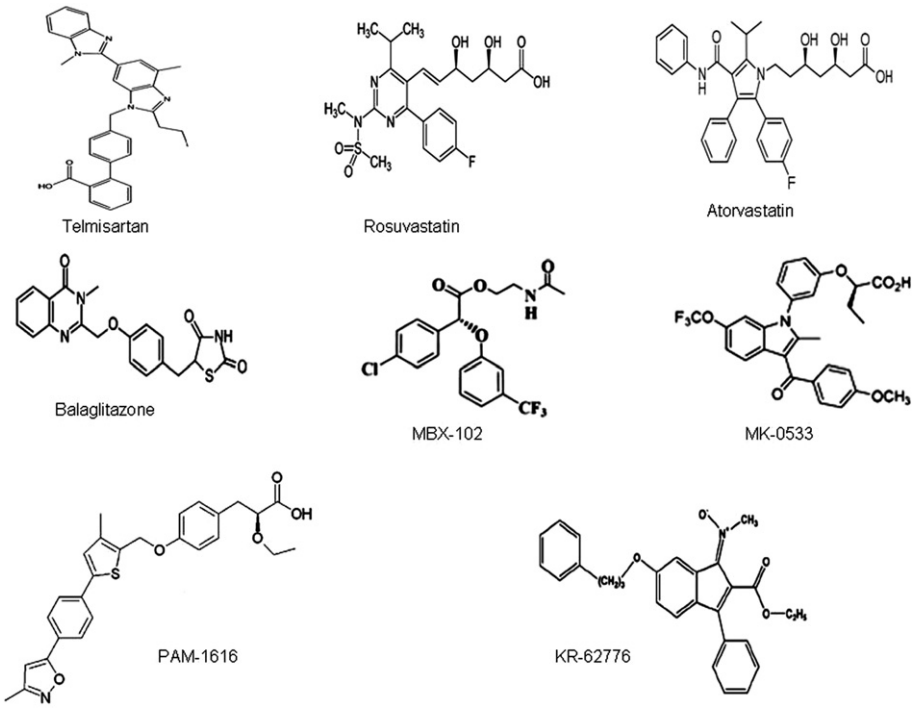

Figure 2

Chemical structures of pharmacological agents that submaximally activate PPARγ.

2008; Yuen *et al.*, 2011). Telmisartan inhibited 9,11-dideoxy-11α,9α-epoxymethanoPGF$_{2\alpha}$ (U46619)- or endothelin-1-induced contraction of mesenteric arteries from male C57BL/6J mice. However, this inhibition was found to be abolished in mesenteric arteries from eNOS-knockout or PPARγ-knockout mice (Yuen *et al.*, 2011). Moreover, telmisartan-induced augmentation of eNOS expression and activation and NO production were reversed upon co-treatment with GW9662, a selective PPARγ antagonist. Interestingly, telmisartan-mediated eNOS activation through PPARγ activation was suggested to be unrelated to it antagonist effects on AT_1 receptors (Yuen *et al.*, 2011). This clearly indicates that telmisartan, through partial activation of PPARγ, thereby subsequently activating eNOS and generating NO in the vascular endothelium, has endothelial protective potential. Wenzel *et al.* (2008) demonstrated that telmisartan therapy reduced vascular oxidative stress, prevented the down-regulation of BH4-synthesizing enzyme (GTP-cyclohydrolase I) expression and inhibited eNOS uncoupling to prevent endothelial dysfunction in experimental diabetes mellitus. Moreover, the phosphorylation of eNOS at Ser^{1177} was noted to be decreased as a result of diabetes mellitus and this was considerably normalized by telmisartan therapy (Wenzel *et al.*, 2008), explaining the beneficial effect of telmisartan in preventing diabetes mellitus-induced VED. A recent study confirmed that the vascular protective potential of telmisartan against diabetes mellitus-associated VED and associated vascular complications is predominantly mediated through its partial PPARγ activating property (Toyama *et al.*, 2011). Telmisartan treatment of apolipoprotein E-deficient mice fed a high-fat diet was shown to significantly reduce blood pressure and considerably improve endothelial dysfunction (as assessed by the vasodilator response to acetylcholine) by modulating eNOS expression in the aorta; an effect that might be due to its action as a partial agonist of PPARγ (Nakagami *et al.*, 2008). Moreover, telmisartan was suggested to enhance the bioavailability and vascular generation of NO through a PPARγ-mediated action (Ikejima *et al.*, 2008). In this study, telmisartan was found to increase acetylcholine-mediated release of NO in genetically hyperlipidaemic rabbits [Watanabe heritable hyperlipidemic (WHHL) rabbits]; an effect that was abolished by co-treatment with the selective PPARγ antagonist, GW9662. Moreover, telmisartan decreased the atherosclerotic plaque area in the thoracic aorta of WHHL rabbits (Ikejima *et al.*, 2008). A recent study revealed that telmisartan could protect against the impaired vasodilatation observed in genetically fatty rats with metabolic syndrome through its anti-oxidative and anti-nitrative stress properties (Kagota *et al.*, 2011). Interestingly, the PPARγ-mediated vascular endothelial protective potential of telmisartan was confirmed by Ono *et al.* (2009) in patients with essential hypertension. Telmisartan improved VED by reducing ADMA, an eNOS inhibitor synthesized by endothelial cells, and decreasing pulse-wave velocity through its additional mechanism associated with PPARγ activation, independent of its AT_1 receptor blockade-mediated hypotensive action (Ono *et al.*, 2009). The leucocyte–endothelial interaction is a fundamental event in the induction and progression of atherosclerotic plaques. Cicha *et al.* (2011) demonstrated that telmisartan has ability to decrease TNF-α-induced recruitment of monocytic cells and endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) in human umbilical

vein endothelial cells. However, the inhibitory effect of telmisartan on monocytic cell recruitment and VCAM-1 induction was found to be attenuated in the presence of the PPARγ antagonist, GW9662, suggesting a key role of PPARγ activation in this process. These authors suggested that this mechanism could possibly contribute to the beneficial effects of telmisartan in protecting atherosclerosis-prone arterial regions (Cicha *et al.*, 2011). It is worth mentioning that telmisartan has the potential to down-regulate the expression of AT_1 receptors both at the mRNA and protein levels in a dose- and time-dependent manner (Imayama *et al.*, 2006). This study, intriguingly, demonstrated that telmisartan-induced down-regulation of AT_1 receptors was mediated through its ability to activate PPARγ (Imayama *et al.*, 2006). Thus, it is possible that submaximal activation of PPARγ could inhibit the detrimental effects of angiotensin-II on the cardiovascular system through down-regulation of AT_1 receptors as well. Therefore, submaximal PPARγ activation could, in fact, be beneficial in preventing cardiovascular abnormalities associated with overactivation of the renin–angiotensin–aldosterone system (RAAS).

Statins are promising agents for improving vascular endothelial function and preventing the pathogenesis of atherosclerosis and hypertension and both-associated cardiovascular disease. We have recently suggested a potential interplay between statins and PPARs in preventing cardiovascular abnormalities (Balakumar and Mahadevan, 2012). It is noteworthy that treatment of mice deficient in LDL receptors and leptin (obese dyslipidemic mice with elevated blood pressure due to NO-sensitive blood pressure variability) with rosuvastatin normalized elevated blood pressure, independently of changes in plasma cholesterol, by up-regulating PPARγ in the aortic arch (Desjardins *et al.*, 2008). However, GW9662 and siRNA raised against PPARγ prevented the blood pressure-lowering effect of rosuvastatin, suggesting that up-regulation of PPARγ in the endothelium could play an additional role in the vasculoprotective effects of rosuvastatin (Desjardins *et al.*, 2008). This study strongly suggested that rosuvastatin regulates blood pressure homeostasis via a PPARγ-NO-dependent mechanism (Desjardins *et al.*, 2008). In addition, the anti-atherogenic effect of atorvastatin in male Japanese rabbits fed high cholesterol was found to be associated with an increase in the expression of PPARγ, enhancement of NO concentration and decrease in plasminogen activator inhibitor-1 level (Yang *et al.*, 2010). These studies suggest that the generation of NO and subsequent endothelial protection induced by statins could be mediated through a PPARγ signalling mechanism. Moreover, we have recently suggested that eNOS could play a pivotal role in mediating statins-associated cardiovascular protection (Balakumar *et al.*, 2012).

Ischaemic pre- and post-conditioning are well-known endogenous cardioprotective methods (Balakumar and Babbar, 2012). It has been suggested that the inhibitory effect of lovastatin on the cardioprotective and infarct size-limiting potentials of ischaemic pre- and post-conditioning (Kocsis *et al.*, 2008) could possibly involve an altered expression pattern of PPARγ (Onody *et al.*, 2003), suggesting a possible adverse cross-talk modulation of PPARγ by statins.

Numerous studies have demonstrated the cardiovascular protective properties of red wine. Red wine polyphenols improved cardiovascular remodelling and vascular function in rats with NO-deficient hypertension. Furthermore, red wine significantly depresses experimental myocardial fibrosis and enhances endothelium-dependent relaxation in the aorta (Bernátová *et al.*, 2002; Pechánová *et al.*, 2004). Interestingly, the ameliorating effects of red wine on cardiometabolic disease have been shown to be partially associated with its ability to activate PPARγ (Zoechling *et al.*, 2011). Some natural substances like curcumin have cardioprotective effects due to a potent anti-oxidant action (Miriyala *et al.*, 2007). Interestingly, curcumin has the ability to activate PPARγ (Jacob *et al.*, 2007). Therefore, it is possible that the PPARγ activating property of curcumin may play a role on its cardioprotective action; however, further studies are needed to prove this contention. Taken together, the pharmacological agents that submaximally activate PPARγ, as listed in Table 1, should be investigated to explore their potential in preventing cardiovascular disorders.

Concluding remarks

Discrepancies have persisted ever since the approval of thiazolidinedione-like PPARγ full agonists for the treatment of T2DM. PPARγ full agonists, indubitably, achieved their clinical target of enhancing insulin sensitivity and reducing hyperglycaemia in patients with T2DM. Unfortunately, these agents have been associated with serious adverse effects, including liver dysfunction, fluid retention, oedema and heart failure, leaving pioglitazone as the only thiazolidinedione suitable for the clinical use. However, the incidence of bladder cancer with pioglitazone has meant that there are now no thiazolidinediones available for clinical use to treat patients with T2DM. Moreover, a rosiglitazone-like full agonist of PPARγ has almost been withdrawn from the market due to its high incidence of cardiovascular side effects such as increased risk of coronary heart disease and heart attack. It seems that the road for full PPARγ agonists has comes to an end due to their undesirable side effects. However, telmisartan, being a partial PPARγ agonist, does not have such adverse side effects, and it has the potential to preventing the dysfunction of vascular endothelium and improve cardiovascular outcomes in patients with cardiovascular and renal abnormalities independently of its classical AT_1 receptor blocking action. Therefore, it is plausible that partial/submaximal activation of PPARγ may have a selective vascular protective action devoid of the undesirable side effects associated with full PPARγ agonists. Furthermore, submaximal activation of PPARγ, by activating eNOS, generating NO, reducing oxidative stress, enhancing the bioavailability of NO and inhibiting the adhesion cascade and vascular inflammation, could have an important endothelial defensive action. The partial activation of PPARγ, as in the case of telmisartan, induces enough of an insulin sensitizing action (which might not be as marked as that of full PPARγ agonists), has a selective action on the regulation of vascular function and improves VED. In addition to telmisartan's submaximal action on PPARγ in preventing VED, another class of drugs, statins, have been shown to have vascular protective potential by a mechanism involving the up-regulation and activation of PPARγ. Therefore, developing a new generation of selective, partial PPARγ agonists that submaximally activate

PPARγ may have important implications for the regulation of vascular endothelial function and may offer new perspectives for the treatment of VED and VED-associated cardiovascular disorders such as atherosclerosis, hypertension and coronary heart disease. In the light of this, an investigation into the vascular protective potentials of pharmacological agents that submaximally activate PPARγ such as balaglitazone, MBX-102, MK-0533, PAR-1622, PAM-1616, KR-62776 and SPPARγM5 (these compounds are devoid of or have a reduced tendency to cause the adverse side effects associated with full PPARγ agonists) is urgently needed.

Acknowledgements

We express our gratitude to Dr Rajendar Singh Sra, MD, Chairman, and Shri Om Parkash, Director, Rajendra Institute of Technology and Sciences, Sirsa, India, for their constant support for this study.

Conflict of interest

No conflict of interest has been declared.

References

Acton JJ 3rd, Akiyama TE, Chang CH, Colwell L, Debenham S, Doebber T *et al.* (2009). Discovery of (2R)-2-(3-{3-[(4-Methoxyphenyl)carbonyl]-2-methyl-6-(trifluoromethoxy)-1H-indol-1-yl}phenoxy)butanoic acid (MK-0533): a novel selective peroxisome proliferator-activated receptor gamma modulator for the treatment of type 2 diabetes mellitus with a reduced potential to increase plasma and extracellular fluid volume. J Med Chem 52: 3846–3854.

Alexander SPH, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th Edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Balakumar P, Babbar L (2012). Preconditioning the hyperlipidemic myocardium: fact or fantasy? Cell Signal 24: 589–595.

Balakumar P, Kaur J (2009). Is nicotine a key player or spectator in the induction and progression of cardiovascular disorders? Pharmacol Res 60: 361–368.

Balakumar P, Mahadevan N (2012). Interplay between statins and PPARs in improving cardiovascular outcomes: a double-edged sword? Br J Pharmacol 165: 373–379.

Balakumar P, Rose M, Ganti SS, Krishan P, Singh M (2007a). PPAR dual agonists: are they opening Pandora's Box? Pharmacol Res 56: 91–98.

Balakumar P, Rose M, Singh M (2007b). PPAR ligands: are they potential agents for cardiovascular disorders? Pharmacology 80: 1–10.

Balakumar P, Kaur T, Singh M (2008a). Potential target sites to modulate vascular endothelial dysfunction: current perspectives and future directions. Toxicology 245: 49–64.

Balakumar P, Sharma R, Singh M (2008b). Benfotiamine attenuates nicotine and uric acid-induced vascular endothelial dysfunction in the rat. Pharmacol Res 58: 356–363.

Balakumar P, Kathuria S, Taneja G, Kalra S, Mahadevan N (2012). Is targeting eNOS a key mechanistic insight of cardiovascular defensive potentials of statins? J Mol Cell Cardiol 52: 83–92.

Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M *et al.* (2004). Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 43: 993–1002.

Bernátová I, Pechánová O, Babál P, Kyselá S, Stvrtina S, Andriantsitohaina R (2002). Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. Am J Physiol Heart Circ Physiol 282: H942–H948.

Bishop-Bailey D (2000). Peroxisome proliferator-activated receptors in the cardiovascular system. Br J Pharmacol 129: 823–834.

Blanquicett C, Kang BY, Ritzenthaler JD, Jones DP, Hart CM (2010). Oxidative stress modulates PPAR gamma in vascular endothelial cells. Free Radic Biol Med 48: 1618–1625.

Budzyn K, Marley PD, Sobey CG (2006). Targeting Rho and Rho-kinase in the treatment of cardiovascular disease. Trends Pharmacol Sci 27: 97–104.

Calnek DS, Mazzella L, Roser S, Roman J, Hart CM (2003). Peroxisome proliferator-activated receptor gamma ligands increase release of nitric oxide from endothelial cells. Arterioscler Thromb Vasc Biol 23: 52–57.

Caramori PR, Zago AJ (2000). Endothelial dysfunction and coronary artery disease. Arq Bras Cardiol 75: 163–182.

Chang CH, McNamara LA, Wu MS, Muise ES, Tan Y, Wood HB *et al.* (2008). A novel selective peroxisome proliferator-activator receptor-gamma modulator-SPPARgammaM5 improves insulin sensitivity with diminished adverse cardiovascular effects. Eur J Pharmacol 584: 192–201.

Chen Z, Peng IC, Sun W, Su MI, Hsu PH, Fu Y *et al.* (2009). AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. Circ Res 104: 496–505.

Chen ZP, Mitchelhill KI, Michell BJ, Stapleton D, Rodriguez-Crespo I, Witters LA *et al.* (1999). AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett 443: 285–289.

Cho DH, Choi YJ, Jo SA, Jo I (2004). Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone: evidence for involvement of peroxisome proliferator-activated receptor (PPAR) gamma-dependent and PPARgamma-independent signaling pathways. J Biol Chem 279: 2499–2506.

Cicha I, Urschel K, Daniel WG, Garlichs CD (2011). Telmisartan prevents VCAM-1 induction and monocytic cell adhesion to endothelium exposed to non-uniform shear stress and TNF-α. Clin Hemorheol Microcirc 48: 65–73.

Desjardins F, Balligand JL (2006). Nitric oxide-dependent endothelial function and cardiovascular disease. Acta Clin Belg 61: 326–334.

Desjardins F, Sekkali B, Verreth W, Pelat M, De Keyzer D, Mertens A *et al.* (2008). Rosuvastatin increases vascular endothelial PPARgamma expression and corrects blood pressure variability in obese dyslipidaemic mice. Eur Heart J 29: 128–137.

Diep QN, Benkirane K, Amiri F, Cohn JS, Endemann D, Schiffrin EL (2004). PPAR alpha activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats. J Mol Cell Cardiol 36: 295–304.

Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999). Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399: 601–605.

Duan SZ, Usher MG, Mortensen RM (2008). Peroxisome proliferator-activated receptor-gamma-mediated effects in the vasculature. Circ Res 102: 283–294.

Dubois M, Vantyghem MC, Schoonjans K, Pattou F (2002). Thiazolidinediones in type 2 diabetes. Role of peroxisome proliferator-activated receptor gamma (PPARgamma). Ann Endocrinol (Paris) 63: 511–523.

Ferdinandy P, Schulz R (2003). Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. Br J Pharmacol 138: 532–543.

Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R (2001). Phosphorylation of Thr(495) regulates Ca(2+)/calmodulin-dependent endothelial nitric oxide synthase activity. Circ Res 88: E68–E75.

Fontana J, Fulton D, Chen Y, Fairchild TA, McCabe TJ, Fujita N *et al.* (2002). Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. Circ Res 90: 866–873.

Forst T, Hanefeld M, Pfützner A (2011). Review of approved pioglitazone combinations for type 2 diabetes. Expert Opin Pharmacother 12: 1571–1584.

Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papap etropoulos A *et al.* (1998). Dynamic activation of endothelial nitric oxide synthase by Hsp90. Nature 392: 821–824.

Govers R, Rabelink TJ (2001). Cellular regulation of endothelial nitric oxide synthase. Am J Physiol Renal Physiol 280: F193–F206.

Gratton JP, Fontana J, O'Connor DS, Garcia-Cardena G, McCabe TJ, Sessa WC (2000). Reconstitution of an endothelial nitric oxide synthase, hsp90 and caveolin-1 complex in vitro: evidence that hsp90 facilitates calmodulin stimulated displacement of eNOS from caveolin-1. J Biol Chem 275: 22268–22272.

Gregoire FM, Zhang F, Clarke HJ, Gustafson TA, Sears DD, Favelyukis S *et al.* (2009). MBX-102/JNJ39659100, a novel peroxisome proliferator-activated receptor-ligand with weak transactivation activity retains antidiabetic properties in the absence of weight gain and edema. Mol Endocrinol 23: 975–988.

Henriksen K, Byrjalsen I, Qvist P, Beck-Nielsen H, Hansen G, Riis BJ *et al.* (2011). BALLET Trial Investigators. Efficacy and safety of the PPARγ partial agonist balaglitazone compared with pioglitazone and placebo: a phase III, randomized, parallel-group study in patients with type 2 diabetes on stable insulin therapy. Diabetes Metab Res Rev 27: 392–401.

Hirata K, Miki N, Kuroda Y, Sakoda T, Kawashima S, Yokoyama M (1995). Low concentration of oxidized low-density lipoprotein and lysophosphatidylcholine upregulate constitutive nitric oxide synthase mRNA expression in bovine aortic endothelial cells. Circ Res 76: 958–962.

Huang PH, Sata M, Nishimatsu H, Sumi M, Hirata Y, Nagai R (2008). Pioglitazone ameliorates endothelial dysfunction and restores ischemia-induced angiogenesis in diabetic mice. Biomed Pharmacother 62: 46–52.

Hwang J, Kleinhenz DJ, Lassègue B, Griendling KK, Dikalov S, Hart CM (2005). Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. Am J Physiol Cell Physiol 288: C899–C905.

Ikejima H, Imanishi T, Tsujioka H, Kuroi A, Kobayashi K, Shiomi M *et al.* (2008). Effects of telmisartan, a unique angiotensin receptor blocker with selective peroxisome proliferator-activated receptor-gamma-modulating activity, on nitric oxide bioavailability and atherosclerotic change. J Hypertens 26: 964–972.

Imayama I, Ichiki T, Inanaga K, Ohtsubo H, Fukuyama K, Ono H *et al.* (2006). Telmisartan downregulates angiotensin II type 1 receptor through activation of peroxisome proliferator-activated receptor gamma. Cardiovasc Res 72: 184–190.

Jacob A, Wu R, Zhou M, Wang P (2007). Mechanism of the anti-inflammatory effect of curcumin: PPAR-gamma activation. PPAR Res 2007: 89369.

Jindal S, Singh M, Balakumar P (2008). Effect of bis (maltolato) oxovanadium (BMOV) in uric acid and sodium arsenite-induced vascular endothelial dysfunction in rats. Int J Cardiol 128: 383–391.

Ju H, Zou R, Venema VJ, Venema RC (1997). Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. J Biol Chem 272: 18522–18525.

Kagota S, Tada Y, Nejime N, Nakamura K, Kunitomo M, Shinozuka K (2011). Telmisartan provides protection against development of impaired vasodilation independently of metabolic effects in SHRSP.Z-Lepr(fa)/IzmDmcr rats with metabolic syndrome. Can J Physiol Pharmacol 89: 355–364.

Kaur J, Reddy K, Balakumar P (2010a). The novel role of fenofibrate in preventing nicotine and sodium arsenite-induced vascular endothelial dysfunction in the rat. Cardiovasc Toxicol 10: 227–238.

Kaur T, Goel RK, Balakumar P (2010b). Effect of rosiglitazone in sodium arsenite-induced experimental vascular endothelial dysfunction. Arch Pharmacal Res 33: 611–618.

Kim MK, Chae YN, Kim HS, Choi SH, Son MH, Kim SH *et al.* (2009a). PAR-1622 is a selective peroxisome proliferator-activated receptor gamma partial activator with preserved antidiabetic efficacy and broader safety profile for fluid retention. Arch Pharm Res 32: 721–727.

Kim J, Han DC, Kim JM, Lee SY, Kim SJ, Woo JR *et al.* (2009b). PPAR gamma partial agonist, KR-62776, inhibits adipocyte differentiation via activation of ERK. Cell Mol Life Sci 66: 1766–1781.

Kim MK, Chae YN, Choi SH, Moon HS, Son MH, Bae MH *et al.* (2011). PAM-1616, a selective peroxisome proliferator-activated receptor γ modulator with preserved anti-diabetic efficacy and reduced adverse effects. Eur J Pharmacol 650: 673–681.

Kobayashi N, Ohno T, Yoshida K, Fukushima H, Mamada Y, Nomura M *et al.* (2008). Cardioprotective mechanism of telmisartan via PPAR-gamma-eNOS pathway in dahl salt-sensitive hypertensive rats. Am J Hypertens 21: 576–581.

Kocsis GF, Pipis J, Fekete V, Kovács-Simon A, Odendaal L, Molnár E *et al.* (2008). Lovastatin interferes with the infarct size-limiting effect of ischemic preconditioning and postconditioning in rat hearts. Am J Physiol Heart Circ Physiol 294: H2406–H2409.

Kota BP, Huang TH, Roufogalis BD (2005). An overview on biological mechanisms of PPARs. Pharmacol Res 51: 85–94.

Kroll J, Waltenberger J (1998). VEGF-A induces expression of eNOS and iNOS in endothelial cells via VEGF receptor-2 (KDR). Biochem Biophys Res Commun 252: 743–746.

Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T *et al.* (2000). Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. Circulation 101: 676–681.

Laufs U, La Fata V, Plutzky J, Liao JK (1998). Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation 97: 1129–1135.

Lebovitz HE, Banerji MA (2001). Insulin resistance and its treatment by thiazolidinediones. Recent Prog Horm Res 56: 265–294.

Li R, Zhang H, Wang W, Wang X, Huang Y, Huang C *et al*. (2010). Vascular insulin resistance in prehypertensive rats: role of PI3-kinase/Akt/eNOS signaling. Eur J Pharmacol 628: 140–147.

Liang C, Ren Y, Tan H, He Z, Jiang Q, Wu J *et al*. (2009). Rosiglitazone via upregulation of Akt/eNOS pathways attenuates dysfunction of endothelial progenitor cells, induced by advanced glycation end products. Br J Pharmacol 158: 1865–1873.

Michel JB, Feron O, Sacks D, Michel T (1997). Reciprocal regulation of endothelial nitric-oxide synthase by Ca21-calmodulin and caveolin. J Biol Chem 272: 15583–15586.

Michell BJ, Griffiths JE, Mitchelhill KI, Rodriguez-Crespo I, Tiganis T, Bozinovski S *et al*. (1999). The Akt kinase signals directly to endothelial nitric oxide synthase. Curr Biol 9: 845–848.

Michell BJ, Chen ZP, Tiganis T, Stapleton D, Katsis F, Power DA *et al*. (2001). Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. J Biol Chem 276: 17625–17628.

Miriyala S, Panchatcharam M, Rengarajulu P (2007). Cardioprotective effects of curcumin. Adv Exp Med Biol 595: 359–377.

Mount PF, Kemp BE, Power DA (2007). Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. J Mol Cell Cardiol 42: 271–279.

Nakagami H, Osako MK, Takami Y, Hanayama R, Koriyama H, Mori M *et al*. (2008). Differential response of vascular hepatocyte growth factor concentration and lipid accumulation between telmisartan and losartan in ApoE-deficient mice. Mol Med Report 1: 657–661.

Naseem KM (2005). The role of nitric oxide in cardiovascular disease. Mol Aspects Med 26: 33–65.

Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES *et al*. (2003). American Heart Association; American Diabetes Association. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Circulation 108: 2941–2948.

Niegowska J, Niegowska M, Jasiński B (2005). Telmisartan in monotherapy of essential hypertension in young men – time of drug administration and 24-hours blood pressure and heart rate. Pol Arch Med Wewn 114: 868–873.

Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P *et al*. (2006). Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. Circ Res 99: 1426–1432.

Noris M, Morigi M, Donadelli R, Aiello S, Foppolo M, Todeschini M *et al*. (1995). Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. Circ Res 76: 536–543.

Ono Y, Nakaya Y, Bando S, Soeki T, Ito S, Sata M (2009). Telmisartan decreases plasma levels of asymmetrical dimethyl-L-arginine and improves lipid and glucose metabolism and vascular function. Int Heart J 50: 73–83.

Onody A, Zvara A, Hackler L Jr, Vígh L, Ferdinandy P, Puskás LG (2003). Effect of classic preconditioning on the gene expression pattern of rat hearts: a DNA microarray study. FEBS Lett 536: 35–40.

Palee S, Chattipakorn S, Phrommintikul A, Chattipakorn N (2011). PPARγ activator, rosiglitazone: is it beneficial or harmful to the cardiovascular system? World J Cardiol 3: 144–152.

Palmer RM, Ashton DS, Moncada S (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333: 664–666.

Patel RR (2009). Thiazolidinediones and congestive heart failure: a judicious balance of risks and benefits. Cardiol Rev 17: 132–135.

Pechánová O, Bernátová I, Babál P, Martínez MC, Kyselá S, Stvrtina S *et al*. (2004). Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. J Hypertens 22: 1551–1559.

Polikandriotis JA, Mazzella LJ, Rupnow HL, Hart CM (2005). Peroxisome proliferator-activated receptor gamma ligands stimulate endothelial nitric oxide production through distinct peroxisome proliferator-activated receptor gamma-dependent mechanisms. Arterioscler Thromb Vasc Biol 25: 1810–1816.

Puddu P, Puddu GM, Zaca F, Muscari A (2000). Endothelial dysfunction in hypertension. Acta Cardiol 55: 221–232.

Quinn CE, Hamilton PK, Lockhart CJ, McVeigh GE (2008). Thiazolidinediones: effects on insulin resistance and the cardiovascular system. Br J Pharmacol 153: 636–645.

Ríos-Vázquez R, Marzoa-Rivas R, Gil-Ortega I, Kaski JC (2006). Peroxisome proliferator-activated receptor-gamma agonists for management and prevention of vascular disease in patients with and without diabetes mellitus. Am J Cardiovasc Drugs 6: 231–242.

Ruilope LM (2011). Telmisartan for the management of patients at high cardiovascular risk. Curr Med Res Opin 27: 1673–1682.

Rush JW, Denniss SG, Graham DA (2005). Vascular nitric oxide and oxidative stress: determinants of endothelial adaptations to cardiovascular disease and to physical activity. Can J Appl Physiol 30: 442–474.

Saltiel AR, Olefsky JM (1996). Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45: 1661–1669.

Schupp M, Janke J, Clasen R, Unger T, Kintscher U (2004). Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. Circulation 109: 2054–2057.

Takahashi S, Mendelsohn ME (2003). Synergistic activation of endothelial nitric oxide synthase (eNOS) by HSP90 and Akt: calcium-independent eNOS activation involves formation of an HSP90-Akt-CaM-bound eNOS complex. J Biol Chem 278: 30821–30827.

Tontonoz P, Hu E, Spiegelman BM (1995). Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr Opin Genet Dev 5: 571–576.

Toyama K, Nakamura T, Kataoka K, Yasuda O, Fukuda M, Tokutomi Y *et al*. (2011). Telmisartan protects against diabetic vascular complications in a mouse model of obesity and type 2 diabetes, partially through peroxisome proliferator activated receptor-γ-dependent activity. Biochem Biophys Res Commun 410: 508–513.

Vaziri ND, Wang XQ (1999). cGMP-mediated negative-feedback regulation of endothelial nitric oxide synthase expression by nitric oxide. Hypertension 34: 1237–1241.

Wakino S, Hayashi K, Kanda T, Tatematsu S, Homma K, Yoshioka K *et al*. (2004). Peroxisome proliferator-activated receptor gamma ligands inhibit Rho/Rho kinase pathway by inducing protein tyrosine phosphatase SHP-2. Circ Res 95: e45–e55.

Wenzel P, Schulz E, Oelze M, Müller J, Schuhmacher S, Alhamdani MS *et al*. (2008). AT1-receptor blockade by telmisartan upregulates GTP-cyclohydrolase I and protects eNOS in diabetic rats. Free Radic Biol Med 45: 619–626.

Wong WT, Tian XY, Xu A, Yu J, Lau CW, Hoo RL *et al*. (2011). Adiponectin is required for PPARγ-mediated improvement of endothelial function in diabetic mice. Cell Metab 14: 104–115.

Wyatt AW, Steinert JR, Mann GE (2004). Modulation of the L-arginine/nitric oxide signalling pathway in vascular endothelial cells. Biochem Soc Symp 71: 143–156.

Xiao Z, Wang T, Qin H, Huang C, Feng Y, Xia Y (2011). Endoplasmic reticulum Ca2+ release modulates endothelial nitric-oxide synthase via extracellular signal-regulated kinase (ERK) 1/2-mediated serine 635 phosphorylation. J Biol Chem 286: 20100–20108.

Yamagishi S, Takeuchi M (2005). Telmisartan is a promising cardiometabolic sartan due to its unique PPAR-gamma-inducing property. Med Hypotheses 64: 476–478.

Yang P, Li Y, Li JJ, Qin L, Li XY (2010). Up-regulating PPAR-γ expression and NO concentration, and down-regulating PAI-1 concentration in a rabbit atherosclerotic model: the possible antiatherogenic and antithrombotic effects of atorvastatin. Int J Cardiol 139: 213–217.

Yu J, Zhang Z, Li Z, Feng X, He L, Liu S *et al*. (2010). Peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist improves coronary artery endothelial function in diabetic patients with coronary artery disease. J Int Med Res 38: 86–94.

Yuen CY, Wong WT, Tian XY, Wong SL, Lau CW, Yu J *et al*. (2011). Telmisartan inhibits vasoconstriction via PPARγ dependent expression and activation of endothelial nitric oxide synthase. Cardiovasc Res 90: 122–129.

Zheng J, Bird IM, Melsaether AN, Magness RR (1999). Activation of the mitogen-activated protein kinase cascade is necessary but not sufficient for basic fibroblast growth factor- and epidermal growth factor-stimulated expression of endothelial nitric oxide synthase in ovine fetoplacental artery endothelial cells. Endocrinology 140: 1399–1407.

Zoechling A, Liebner F, Jungbauer A (2011). Red wine: a source of potent ligands for peroxisome proliferator-activated receptor γ. Food Funct 2: 28–38.