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Therapeutical effects of PPAR agonists assessed by biomarker modulation

G. CHINETTI-GBAGUIDI, J.-C. FRUCHART, & B. STAELS

UR 545 INSERM, Institut Pasteur de Lille and Université de Lille 2, Lille, France

Abstract
The metabolic syndrome is defined as the clustering of cardiovascular risk factors, such as glucose intolerance, hyperinsulinemia, dyslipidemia, coagulation disturbances and hypertension. Activators of the nuclear receptors peroxisome proliferator-activated receptors (PPARs) modulate several of the metabolic risk factors pre-disposing to atherosclerosis. Fibrates are hypolipidemic drugs operating through activation of PPARα, whereas glitazones are insulin sensitizers activating PPARγ. In addition, these drugs exert pleiotropic and anti-inflammatory actions. This review will focus on the different effects of fibrates and glitazones, as measured by biomarker modulation, on the development of atherosclerosis and cardiovascular disease.

Keywords: *Nuclear receptors, fibrates, glitazones, metabolic syndrome*

Introduction

The metabolic syndrome, which is characterized by the simultaneous presence of one or more metabolic disorders, such as glucose intolerance, hyperinsulinemia, dyslipidemia, coagulation disturbances and hypertension, is defined as the clustering of cardiovascular risk factors with insulin resistance and android obesity (Figure 1). Activators of peroxisome proliferator-activated receptors (PPARs) modulate several of these metabolic risk factors pre-disposing to atherosclerosis.

The PPAR family is constituted of PPARα, PPARγ and PPARβ/δ, each showing a distinct tissue distribution pattern. PPARα is expressed preferentially in tissues where fatty acids are catabolized, whereas PPARγ is highest expressed in adipose tissue where it modulates crucial aspects of adipocyte differentiation and lipid metabolism, thus impacting on glucose metabolism. PPARβ/δ is expressed at high levels in muscle and intestine, but is also present in adipose tissue and liver. All PPARs are expressed in most cell types of the vascular wall, including endothelial cells, monocyte/macrophages, smooth muscle cells and lymphocytes, as well as in atherosclerotic plaques. PPARs regulate lipid and lipoprotein metabolism and glucose homeostasis after activation by fatty acids and their metabolites or, under therapeutic conditions, by synthetic ligands, such as the hypolipidemic fibrates (low affinity PPARα activators) and the anti-diabetic glitazones (a class of high affinity PPARγ ligands). Most of the physiological functions of PPARs can be explained by their activity as transcription

Correspondence: Giulia Chinetti-Gbaguidi, UR 545 INSERM, Institut Pasteur de Lille, 1, rue Calmette BP245, 59019 Lille, France. Tel: 33-3-20-87 71 70. Fax: 33-3-20-87 71 98. Email: giulia.chinetti@pasteur-lille.fr

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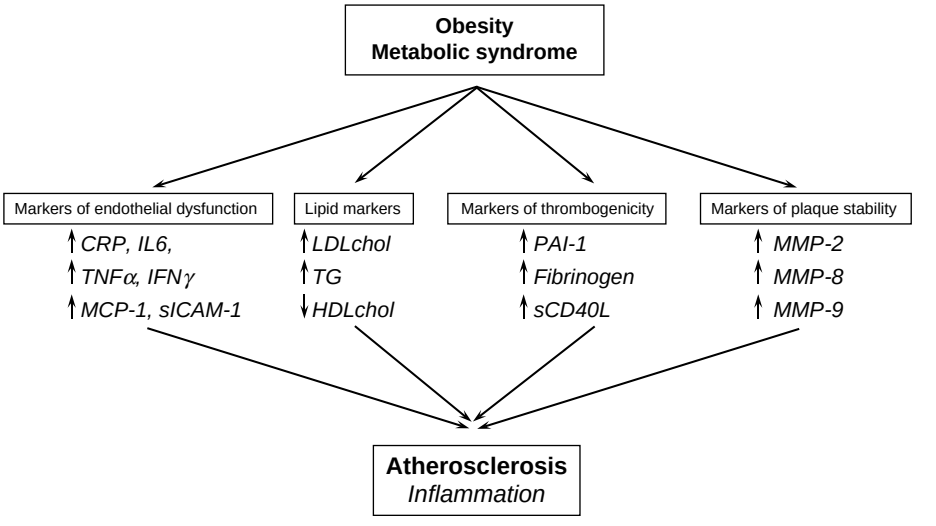

Figure 1. Biomarkers associated with features of the metabolic syndrome. The metabolic syndrome is defined as the clustering of cardiovascular risk factors pre-disposing to atherosclerosis development, such as glucose intolerance, hyperinsulinemia, dyslipidemia, coagulation disturbances and hypertension.

factors, modulating the expression of specific target genes. Upon ligand activation, PPARs regulate gene transcription by dimerizing with the Retinoid X Receptor (RXR) and binding to specific PPAR response elements (PPREs) within the regulatory regions of target genes (Chinetti et al. 2003). PPARs can also repress gene transcription in a DNA binding-independent manner by negatively interfering with other signalling pathways via protein–protein interactions and co-factor competition (Chinetti et al. 2003). Such trans-repression mechanism is likely to participate in the anti-inflammatory actions of PPARs.

PPAR agonists and metabolic effects

In humans, activation of PPARα decreases plasma triglycerides (TG) and increases HDL cholesterol levels. The TG-lowering effects are the consequence of actions on different metabolic pathways (Staels et al. 1998a). Fibrates enhance intra-vascular triglyceride lipolysis by inducing hepatic LPL expression and activity and increasing accessibility of TG-rich lipoproteins due to reduced apoCIII content (Staels et al. 1998a). Fibrates increase FA catabolism and reduce FA synthesis, resulting in a limited TG and VLDL production in the liver. Interestingly, expression of apoAV, a recently identified determinant of plasma TG levels, is induced by fibrates in human hepatocytes (Vu-Dac et al. 2003). As a consequence of their action on lipoprotein lipolysis, these drugs also change plasma LDL composition by reducing the levels of small dense LDL, which possess lower affinity for the LDL-receptor than large LDL, thus accelerating LDL catabolism. Fibrates also influence HDL metabolism via PPARα by increasing hepatic gene transcription of apoAI and apoAII (Staels et al. 1998a). PPARα agonists directly stimulate the first steps of the reverse cholesterol transport pathway, namely the efflux of cholesterol from peripheral cells through the induction of a number of trans-membrane receptors, such as the scavenger receptor SR-BI/CLA-1 and the ATP-binding cassette (ABC)A1 in macrophages (Chinetti et al.

2000a, 2001a). Glitazones are anti-diabetic drugs that reduce glucose and HbA1c levels in type 2 diabetic patients. In adipose tissue, PPARγ activation induces the expression of genes controlling FA metabolism, such as LPL, CD36 and acyl-CoA synthetase, resulting in an increased FA uptake and TG storage in adipocytes, thus diverting FA away from skeletal muscle and liver and relieving their inhibitory effect on glucose metabolism and oxidation. In addition, PPARγ agonists improve adipocyte insulin sensitivity and stimulate, at least in rodents, glycerol kinase activity, thus increasing the incorporation of glycerol into TG and diminishing the release of free FA by adipocytes. These effects contribute to the lowering of circulating FA levels, a biomarker of therapeutic efficacy. Analysis of the lipid profile of patients treated with glitazones demonstrated that these drugs significantly increase plasma HDL levels (Sunayama et al. 1999, Lawrence et al. 2004) and reduce small dense LDL (Hirano et al. 1998, Tack et al. 1998) (Figure 2). Since increased levels of HDL are associated with decreased risk of atherosclerosis, PPAR agonists might provide a new approach for the treatment of cardiovascular disease by increasing HDL levels and promoting reverse cholesterol transport. The influence of fibrates on cardiovascular risk has been demonstrated in a number of primary and secondary prevention as well as coronary angiography trials (LOCAT, BECAIT, VAHIT, DAIS, . . .) (Chinetti et al. 2003). The beneficial effects of glitazones on cardiovascular mortality in type 2 diabetic patients is currently assessed in several prospective intervention trials, such as the RECORD, PROACTIVE and DREAM trials.

PPARs and inflammation control

The effects of PPAR agonists in the control of the inflammatory response have been observed in a variety of cell types. Activated PPARα inhibits the production of inflammatory response markers, such as endothelin-1, ICAM-1, VCAM-1 in endothelial cells and tissue factor, matrix metalloproteinase (MMP)-9 and TNFα in macrophages (reviewed in Chinetti et al. 2000b). In human aortic smooth muscle cells (SMC), PPARα activation inhibits IL-1-stimulated IL-6 secretion and decreases IL-6 and COX2 gene transcription (Staels et al. 1998b). The role of PPARγ in inflammation is more controversial due to the fact that PPARγ-deficient mice are not viable and genetic evidence for such activity is, thus, difficult to obtain. Nevertheless, PPARγ ligands clearly inhibit TNFα, IL-6 and IL-1β expression in monocytes, IFNγ-induced expression of the T-cell-specific CXC-chemokines, IFNγ-inducible protein 10 (IP-10), Mig, I-TAC and cell-adhesion molecules and endothelin-1 in endothelial cells (Chinetti et al. 2001b). In macrophages, PPARγ ligands repress inducible nitric oxyde synthase (iNOS) and MMP-9 expression (Chinetti et al. 2001b) and increase the production of the IL-1 receptor antagonist (Meier et al. 2002). PPARs are also expressed in immune cells (Faveeuw et al. 2000, Gosset et al. 2001) where they modulate the expression of cytokines and co-stimulatory molecules. In dendritic cells (DC), PPARγ activators reduce the secretion of IL-12 (Faveeuw et al. 2000, Gosset et al. 2001), a pro-inflammatory and pro-atherogenic cytokine and affect the surface expression of co-stimulatory molecules, such as CD80 and CD86, and the synthesis of chemokines, including RANTES and IP-10 (Gosset et al. 2001). In T-cells, PPARγ activators reduce the synthesis of IFNγ and TNFα (Marx et al. 2002) and decrease production of IL-2 by negatively interfering with the T-cell specific transcription factor NFAT (Yan et al. 2000).

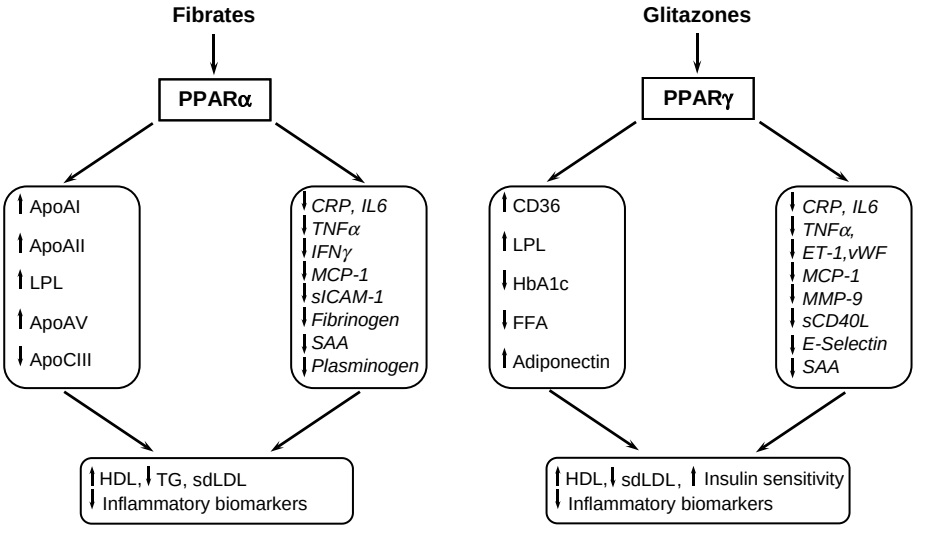

Figure 2. Effects of PPARα and PPARγ activators on biomarker modulation. Fibrates, through PPARα activation, regulate the expression of genes involved in FA oxidation, TG synthesis and lipolysis and HDL metabolism. Glitazones, PPARγ activators, primarily interfere with insulin signalling, FA metabolism and cytokine production. As a consequence, fibrates decrease plasma TG and small dense LDL (sdLDL) and increase HDL levels, while glitazones improve insulin sensitivity. Both drug classes reduce blood concentrations of inflammatory biomarkers, suggesting an overall beneficial effect of PPARα and PPARγ agonists in correcting the global risk profile pre-disposing to cardiovascular disease.

Moreover, PPARγ ligands can also influence major histocompatibility complex class (MHC) II-mediated T-cell activation by inhibiting IFNγ-induced MHC-II expression in vascular cells (Kwak et al. 2002). By contrast, the actions of PPARα in T-cells are less well documented. In human T-cells, PPARα activators reduce the secretion of IFNγ, TNFα and IL-2 (Marx et al. 2002). PPARα is highly expressed in liver, where it has initially been thought to mainly regulate genes involved in fatty acid β-oxidation (Chinetti et al. 2000b). However, in primary human hepatocytes and hepatoma cells, PPARα activators suppress IL-1-induced C-reactive protein (CRP) and IL-6-induced fibrinogen expression, the major acute phase response (APR) proteins in humans (Kleemann et al. 2002), whose plasma concentrations are elevated not only in acute but also in chronic inflammatory states, such as atherosclerosis. This anti-inflammatory action of PPARα is not restricted to these genes, but applies more generally to other APR genes, such as serum amyloid A (SAA) and fibrinogen-α and β (Gervois et al. 2001, 2004).

PPARs and biomarker modulation

Clinical trials using fibrates and glitazones also provide indications regarding the clinical efficacy of PPAR agonists in the control of inflammation. In patients with angiographically established atherosclerosis, fenofibrate treatment decreased the circulating levels of IL-6 and lowered the plasma levels of CRP and fibrinogen (Staels et al. 1998b, Gervois et al. 2004). In obese patients with atherogenic dyslipidemia, gemfibrozil administration also produced a significant reduction of CRP levels (Despres et al. 2003). In addition, fenofibrate treatment significantly reduced plasma

IFNγ and TNFα levels in patients with type IIb hyperlipoproteinemia (Madej et al. 1998). Similar effects were observed in hypertriglyceridemic patients after bezafibrate administration (Jonkers et al. 2002). Fenofibrate treatment also led to a reduction in ICAM-1, MCP-1 and α2-macroglobulin and plasminogen plasma levels in patients with hyperlipoproteinemia (Kowalski et al. 2003, Gervois et al. 2004) (Figure 2).

Treatment of type 2 diabetic patients with rosiglitazone significantly reduced C-reactive protein levels as well as white blood cell count and MMP-9 serum levels (Haffner et al. 2002). In non-diabetic coronary artery disease patients, rosiglitazone treatment resulted in a significant reduction of E-selectin, von Willebrand factor and fibrinogen as well as P-selectin levels and circulating platelet activity (Nakamura et al. 2000, Sidhu et al. 2003, 2004), while in obese patients, rosiglitazone reduced plasma concentrations of MCP-1 and TNFα, an effect already observed after 2 weeks of treatment (Mohanty et al. 2004).

In addition, in patients with coronary artery disease and type 2 diabetes, rosiglitazone significantly reduced SAA levels after only 2 weeks of treatment and decreased TNF-α levels after 6 weeks, thus suggesting an effect independent of the metabolic changes induced by the treatment (Marx et al. 2003a). Patients with type 2 diabetes exhibit elevated levels of other inflammatory markers such as sCD40L, MMP-2, MMP-3 and MMP-9, likely reflecting the increased cardiovascular risk observed in these patients (Marx et al. 2003a,b). Rosiglitazone reduced sCD40L levels as well as MMP-9 as early as 2 weeks after the initiation of treatment (Marx et al. 2003b). Interestingly, it has been reported that the maximal glucose-lowering effect of glitazones is observed only after 8–12 weeks (Raskin et al. 2000). Thus, the difference in the timing of reduction of sCD40L or MMP-9, on the one hand, and glucose, on the other hand, strongly suggests that glitazones might directly affect levels of these biomarkers, independent of their metabolic action.

Conclusions

Considerable evidence indicates that PPARα and PPARγ have beneficial effects in dyslipidemic and T2DM patients, as reflected by a modulation of inflammatory and cardiovascular disease biomarkers. The changes in these biomarkers reflect the role of PPARs as master regulators of lipid and lipoprotein metabolism, glucose homeostasis and the inflammatory response. Although the molecular mechanisms are not yet fully established and the complexity of the regulated systems is important, PPARs appear to interfere at different steps of atherogenesis by blocking vascular cell recruitment, by modulating foam cell formation, by interfering with the inflammatory response and by inhibiting fibrous plaque development and possibly influencing plaque stability and rupture.

Based on these findings, mainly coming from pre-clinical models as well as the first clinical reports measuring different biomarkers, fibrates, glitazones, as well as mixed PPARα/PPARγ ligands, can be expected to have a high potential in the treatment of inflammation-related disorders.

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