The clinical significance of PPARα and γ agonism

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Abstract

The thiazolidinediones are synthetic ligands for nuclear peroxisome proliferator activated receptors (PPARs). PPARγ is a transcription factor, which in adipose tissue promotes adipocyte differentiation and also induces apoptosis of terminally differentiated insulin-resistant adipocytes. This promotes the appearance of smaller insulin-sensitive cells. PPARγ activation also stimulates the genes controlling triglyceride lipolysis, fatty acid uptake and storage in adipose tissue. It induces a diversion of fatty acids away from muscle and influences the expression of adipocytokines leading to improved insulin signalling in muscle and liver. It may also regulate genes involved in insulin signalling. These all result in an increase in insulin sensitivity. PPARγ is also expressed in atherosclerotic lesion foam cells and its activation may exert anti-inflammatory actions and stimulate expression of genes involved in the reverse cholesterol transport pathway. Thiazolidinediones also improve lipoprotein metabolism and this activity is most pronounced for agents that activate PPARα, such as may be the case for pioglitazone.

Key words: adipocytes, atherosclerosis, insulin resistance, PPAR receptors, pioglitazone, thiazolidinedione.

Introduction

As more has been discovered about the peroxisome proliferator-activated receptors (PPARs), it has become increasingly clear that they are prime targets for treatment of the metabolic syndrome. Metabolic syndrome is the cluster of cardiovascular risk factors associated with insulin resistance, including dyslipidaemia, glucose intolerance, hyperinsulinaemia and hypertension (figure 1). The PPARs are important modulators of lipid and glucose metabolism and inflammation. Hence, drugs that can bind with the PPARs have the potential to affect these important cardiovascular risk factors. Although there are three known subtypes of PPAR (α, β/δ and γ) only PPARα and PPARγ are well understood. PPARα is highly expressed in tissues that have a high rate of fat oxidation and it drives fatty acid catabolism in liver, muscle and other tissues. PPARγ is involved in adipocyte differentiation and function, fatty acid uptake and conversion and storage as triglycerides in adipose tissue. Further, both PPARs appear to play a role in inflammatory control. This paper will examine the potential mechanisms whereby PPAR agonists might benefit cardiovascular risk factors in patients with metabolic syndrome.

PPARγ

The only PPARγ agonists currently available are the thiazolidinediones pioglitazone and rosiglitazone. These agents are indicated for type 2 diabetes in which condition they lower hyperglycaemia and hyperinsulinaemia by increasing peripheral insulin sensitivity. The increase in insulin sensitivity is probably largely mediated by the effects of PPARγ on differentiation of adipocytes and the insulin sensitivity of mature adipocytes.

In adipogenesis, adipogenic stimuli (eg. insulin) activate the transcription factor C/EBPβ, which in turn activates PPARγ. PPARγ binds with 9-cis-retinoic acid receptor (RXR) and this complex, both alone and in cooperation with C/EBPα, then stimulates the expression of adipocyte-specific genes, including adipocyte fatty acid binding protein (aP2), lipoprotein lipase (LPL) and acyl-CoA synthetase (ACS).

Stimulation of PPARγ by thiazolidinediones, therefore, promotes adipogenesis and this has been shown in vitro by enhanced insulin-regulated differentiation (monitored by the rate of lipogenesis or triglyceride accumulation) of the
preadipocyte cell-line, 3T3-L1 cells, following incubation with thiazolidinediones, such as pioglitazone. Concomitantly, an increased rate of glucose transport and increased activity of lipogenic enzymes and increased GLUT-4, LPL, and glucose-6-phosphate dehydrogenase mRNA is observed. This latter effect may partly explain why adipocyte differentiation is associated with a lowering of insulin resistance. Increased GLUT-4 will enhance glucose uptake, and increased glucose-6-phosphate dehydrogenase will enhance glucose utilisation.

In the clinical setting, PPARγ stimulation by thiazolidinediones results in weight gain due to a combination of increased fat mass and body water. However, despite the link between obesity and insulin resistance, the weight gain seen with the thiazolidinediones is, on the contrary, associated with a decrease in insulin resistance. This may be because these agents increase subcutaneous fat but decrease visceral fat. Miyazaki et al. showed that patients treated with pioglitazone had a significant increase in subcutaneous fat (p<0.01) and decrease in visceral fat (p<0.05). The decrease in visceral fat with pioglitazone was correlated with a significant improvement in hepatic insulin resistance (R=0.055, p<0.01).

Mature adipocytes secrete a variety of substances that are relevant to the metabolic syndrome. For example, leptin, plasminogen activator inhibitor 1 (PAI-1), adiponectin and tumour necrosis factor α (TNFα) interact with insulin signalling pathways and affect muscle glucose disposal, while angiotensinogen is linked to hypertension. Increased TNFα production may induce insulin resistance by interference with the insulin signalling cascade. Thiazolidinediones have been shown to reduce TNFα expression. Leptin may interfere with insulin signalling and, again, thiazolidinediones reduce leptin expression.

PPARγ agonism may also affect insulin resistance by an effect on free fatty acid (FFA) clearance. Increased fatty acid concentrations are known to decrease glucose metabolism in the muscle. Thiazolidinediones induce LPL and fatty acid transporter protein (FATP) in adipose tissue but not muscle tissue, resulting in increased FFA clearance by adipose tissue without increased FFA reaching the muscle (‘fatty acid steal’). Thiazolidinediones may also reduce adipose tissue hormone-sensitive lipase (HSL) and therefore, limit adipose tissue fatty acid release. Whether one or both of these mechanisms apply, the resulting decreased availability of fatty acids for uptake by muscle improves insulin sensitivity in that tissue via the Randle (glucose-fatty acid cycle) effect (figure 2).

PPARα
The fibrates are synthetic ligands for PPARα. These agents are well-known for their lipid-lowering activities. They raise high-density lipoprotein (HDL) cholesterol and lower triglyceride levels and reduce the concentration of the highly atherogenic, small, dense low-density lipoprotein (LDL) particles. Certain thiazolidinediones, such as pioglitazone, have been suggested to have some degree of action at PPARα. Interestingly, pioglitazone, like fibrates, increases HDL cholesterol and lowers triglycerides, an effect which is not seen with rosiglitazone, a pure PPARγ agonist. Thus, it is tempting to speculate that differences in PPARα activation explain the differential effects of these thiazolidinediones on lipid metabolism. Further studies are required to prove unequivocally this concept.

In the liver, PPARα activation results in increased mitochondrial fatty acid β-oxidation via an increase in FATP, ACS and CPT-1 mRNA, which in turn decreases the amount of triglycerides produced. Further, PPARα increases clearance of triglycerides via a decrease in apolipoprotein (apo) C-III production. Together these effects reduce circulating triglycerides.

PPARα is also important in the control of HDL particle production. PPARα activation induces transcription of the apoA-I and apoA-II genes, thereby increasing HDL cholesterol production. These apolipoproteins are involved in the reverse cholesterol transport pathway which protects against coronary artery disease by causing cholesterol to be effluxed from macrophage-derived foam cells in atherosclerotic plaques into HDL particles (figure 3). We have recently shown that PPARα activation leads to inhibition of acylCoA acyl transferase in the macrophage leading to enhanced release of free cholesterol from esterified cholesterol (unpublished observations). The free cholesterol can then efflux from the macrophage through specific transporter pathways, such as that mediated by the ATP-binding cassette transporter (ABCA1). This ABCA1 protein is also controlled by PPARα and γ. PPARα activation, therefore, improves the reverse cholesterol transport pathway.

PPARα and γ and atherosclerosis
PPARα and γ are expressed in atherosclerotic lesions in human coronary arteries and are believed to be involved in several aspects of atherogenesis. Monocyte differentiation into macrophages and their subsequent transformation into foam cells after accumulating lipids from oxidised LDL are key steps in atherogenesis. These foam cells not only take up lipids but also secrete a variety of inflammatory mediators, such as leukotrienes, prostaglandins and cytokines.

PPARα is present in freshly isolated monocytes and its expression increases during differentiation into macrophages, while PPARγ is only present upon differentiation.
are also expressed in endothelial cells and smooth muscle cells in the atherosclerotic lesion.

As well as stimulating the reverse cholesterol pathway in the macrophage-derived foam cells (as described above), the PPARs may inhibit lipid accumulation and cause foam cell apoptosis. Further, activation of PPARα and γ seems to negatively interfere with several inflammatory signaling pathways, such as the NFκB, the STAT and the AP-1 pathways – pathways that are highly activated in atherosclerotic lesions. This inhibits the production of several cytokines, including TNFα, interleukin 1β (IL-1β) and IL-6. Indeed, the PPARα agonist, fenofibrate, has been shown to reduce IL-6 levels in patients with coronary artery disease, and as mentioned above, thiazolidinediones have been shown to inhibit TNFα production.

PPARα agonists also affect the inflammatory mediators produced by the liver, such as C reactive protein (CRP) and fibrinogen, both of which are known cardiovascular risk factors. Treatment with fenofibrate also reduced the plasma levels of these acute phase proteins in patients with coronary artery disease. Other aspects of the metabolic syndrome that may be modified by PPAR agonists include procoagulant state and microalbuminuria. Changes that favour thrombosis and interfere with fibrinolysis are often seen in individuals with insulin resistance. One factor that is implicated in this is plasminogen activator inhibitor type 1 (PAI-1), which is produced by endothelial cells and adipocytes and interferes with the conversion of plasminogen to plasmin. Levels of PAI-1 are correlated with the risk for myocardial infarction and vascular thrombosis. Treatment of type 2 diabetes patients with troglitazone has been shown to decrease plasma PAI-1.

Microalbuminuria is an important predictor of cardiovascular disease. In streptozotocin-induced diabetic rats, troglitazone was able to prevent diabetic glomerular hyperfiltration and albuminuria as well as increasing mRNA expression of extracellular matrix proteins and transforming growth factor β1 in the glomeruli. Rosiglitazone has been shown to decrease albuminuria in patients with type 2 diabetes.

There are some preliminary studies suggesting that the thiazolidinediones may inhibit atherosclerosis in humans. Koshiyama et al. observed a significant decrease in the common carotid arterial intimal and medial complex thickness (IMT) after six months’ administration of pioglitazone in patients with type 2 diabetes. Similar results were found with troglitazone. In another study with troglitazone, Takagi et al. examined neointimal tissue proliferation following coronary stent implantation in patients with type 2 diabetes. Serial intravascular ultrasound assessment revealed a significantly greater lumen area and smaller intimal area at follow-up in patients treated with troglitazone than controls.

A wealth of emerging data shows anti-atherosclerotic effects of thiazolidinediones in animal models. The apo-E knock out mouse is a well-established animal model of atherosclerosis. These mice are hypercholesterolaemic and spontaneously develop severe atherosclerosis with widespread, fibrous plaque-like lesions. In this mouse model, administration of a dual agonist of PPARα and γ for 11 weeks resulted in a significant reduction (32.4%, p<0.001) in lesion area (figure 4). Troglitazone also significantly inhibited fatty streak lesion formation in this mouse model, with a reduction in lesion area of 45% (p<0.05). In addition, troglitazone and rosiglitazone both inhibited lesion formation in another mouse model of atherosclerosis, the LDL receptor-deficient mouse.

There are also some studies indicating that the thiazolidinediones can reduce cardiovascular risk by affecting blood pressure and vascular resistance. Troglitazone and rosiglitazone significantly decrease blood pressure in hypertensive and non-hypertensive patients with type 2 diabetes and obese patients without diabetes. This appears to occur by improved vasodilatation possibly via PPARγ effects on endothelin 1 expression.
PPARγ agonists reduce insulin resistance primarily through effects in adipose tissue

PPARγ agonists also affect insulin sensitivity directly by affecting insulin signalling pathways

PPARγ agonists indirectly increase insulin sensitivity in skeletal muscle by diverting fatty acid uptake away from muscle and into adipose tissue

PPARα agonists lower circulating triglycerides and raise HDL cholesterol by effects in the liver

Agonists that act at both PPARα and PPARγ may be able to reduce atherogenic lesion size by affecting lipid efflux from foam cells into HDL particles and by reducing inflammatory mediator production

Key messages

Conclusion

We now know that PPARα and γ agonists together can control a number of the metabolic perturbations that are associated with insulin resistance and the metabolic syndrome. PPARα agonists play an important role in the control of dyslipidaemia and also influence a number of haemostatic and coagulation parameters. PPARγ agonists, mainly through their action on adipose tissue influence insulin resistance. Finally, these PPARs also play an important role in atherosclerosis. The greatest cardiovascular benefit in Type 2 diabetes is, therefore, likely to be gained by targeting both PPARα and PPARγ.

References