

PPAR γ – A NEW CONCEPT OF TREATMENT?

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SUMMARY

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily, which also includes the steroid and thyroid hormone receptors. PPARs modulate genes that regulate lipid and glucose metabolism and control many cellular and metabolic processes. Three isotypes called PPAR α , PPAR β/δ and PPAR γ have been identified. Their activity can be modulated by drugs such as fibrates and thiazolidinediones. Understanding the biology and identifying small molecule modulators of the PPARs is an active area of research and may impact chronic diseases such as type 2 diabetes, dyslipidemia, atherosclerosis and the metabolic syndrome.

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INTRODUCTION

Type 2 diabetes mellitus is one of the most common chronic diseases and affects about 5% of adults worldwide. Both insulin resistance and impaired β -cell function are major features of the pathophysiology of type 2 diabetes.

Since their development, PPAR agonists have emerged as an important target for the treatment of insulin resistance and dyslipidemia. Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor family of ligand-activated transcription factors (1). The term PPAR derives from observations that certain compounds such as fibrates could induce the growth of proliferation of peroxisomes, intracellular organelles in rodents. Peroxisomes contain oxidizing enzymes called peroxidases. PPARs control many cellular and metabolic processes and play essential roles in the regulation of cellular differentiation, proliferation and metabolism (glucose, lipid and protein). Nuclear receptors represent novel targets for the development of therapeutic agents for the treatment of metabolic disorders such as type 2 diabetes, dyslipidemia, atherosclerosis and the metabolic syndrome.

MOLECULAR MECHANISMS OF PPARs

PPARs are ligand-dependent intracellular proteins that stimulate transcription of specific genes by binding to specific DNA sequences following activation by the appropriate ligand. The PPARs heterodimerize with the retinoid X receptor (RXR), forming a complex that interacts with specific PPAR response elements (PPREs) within the promoter region of target genes, thereby regulating gene function, which may repress or activate gene expression. When activated by agonist ligand binding, this heterodimer complex recruits transcription coactivators and regulates the transcription of genes involved in the control of lipid and glucose metabolism. Three subtypes called PPAR α , PPAR β/δ and PPAR γ have been identified. The expression of PPAR α , β/δ and γ varies widely from tissue to tissue. PPAR α is expressed in tissues where fatty acid catabolism is important (liver, kidney, heart, muscle, and others) (2). PPAR β/δ is expressed ubiquitously but markedly in brain, adipose tissue and skin. PPAR γ is expressed in three forms: γ 1 is expressed in all tissues, including heart, muscle, kidney, pancreas, colon and spleen; γ 2 is expressed predominantly in adipose tissue; and γ 3 is expressed in white adipose tissue, macrophage and large intestine. PPAR γ is expressed in adipose tissue and controls adipocyte differentiation and lipid storage. They display differential tissue distribution and each of the three subtypes fulfills specific functions; however, all three PPARs affect energy homeostasis and inflammatory responses (3,4).

PPAR LIGANDS

PPAR α , γ and β/δ subtypes have significant differences in their ligand and gene specificities. PPAR α is activated by polyunsaturated fatty acids and by fibrate drugs, and regulates genes that are involved in lipid and lipoprotein metabolism. PPAR α activation increases high density lipoprotein (HDL) cholesterol synthesis and reduces triglycerides. Fibrates are used clinically to treat patients with hypertriglyceridemia (5). Until recently, the biological role of PPAR β/δ remained unclear. PPAR β/δ plays a role in lipid metabolism by stimulating fatty acid oxidation in heart and skeletal muscle cells. Treatment of obese animals

by specific PPAR δ agonists results in normalization of metabolic parameters and reduction of adiposity. PPAR β/δ agonists inhibit cardiomyocyte hypertrophy, normalize lipid status in obese animals and have been proposed as a putative pharmacological target for the management of obesity, dyslipidemia and insulin resistance. PPAR γ is activated by fatty acid derivatives, prostaglandin derivatives and thiazolidinedione drugs (4,6).

Thiazolidinediones are insulin-sensitizing agents that decrease peripheral insulin resistance, increase glucose disposal in muscle and suppress gluconeogenesis in the liver, thereby reducing blood glucose levels in patients with type 2 diabetes. They are used for the treatment of type 2 diabetes and are highly selective PPAR γ agonists. Rosiglitazone and pioglitazone are part of a class of thiazolidinediones antidiabetic agents that improve glucose utilization without stimulating insulin release (7,8).

PPAR γ MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

PPAR γ is a key regulator of glucose homeostasis and adipogenesis. PPAR γ activation results in insulin sensitization and antidiabetic action. Agonists of PPAR, including thiazolidinediones, stimulate adipogenesis and expression of adipocyte-specific genes in fibroblasts, preadipocytes or myocytes that express PPAR γ endogenously or ectopically. The binding to PPAR γ in adipose tissue promotes adipocyte differentiation, resulting in an increase in the number of small, insulin-sensitive adipocytes and an associated decrease in serum-free fatty acid levels and tumor necrosis factor α (TNF α) expression. PPAR γ is known to regulate many genes involved in insulin signaling, such as those that control the expression of the proinflammatory cytokine, TNF α . PPAR γ activation significantly reduces the production of TNF α by adipocytes, which plays an established role in the development of insulin resistance (9,10). Effects of PPAR γ agonists *in vitro* and in animal models provide evidence for additional potential antiatherosclerotic benefits. Thiazolidinediones, *via* binding to the PPAR γ response element in the promoter region of the adiponectin gene, activate

adiponectin gene transcription, increasing plasma adiponectin levels. The increase in insulin sensitivity effected by thiazolidinediones is probably mediated, at least in part, through an increase in plasma adiponectin (11). The adipose protein, resistin, is present in high concentration in obese individuals and appears to impair glucose tolerance. Thiazolidinediones reduce resistin expression in adipose tissue and decrease the synthesis of resistin. Leptin is highly correlated with adiposity, while the activation of PPAR γ is known to inhibit Lep gene expression and leptin release (12). PPAR α and PPAR γ inhibit the expression of inflammatory genes, such as cytokines, matrix metalloproteinases (MMPs), and acute phase proteins. All available data indicate that the activation of PPAR α and PPAR γ modulates oxidative stress-sensitive pathways, redox-responsive nuclear factor- κ B (NF- κ B), activator protein-1 (AP-1), and signal transducers and activators of transcription (STAT). PPAR γ agonists also participate in the control of inflammation in modulating the production of inflammatory mediators, in part by inhibiting the activation of NF- κ B. PPAR γ activators suppress the expression of type I angiotensin II receptor (AT-R1) at the level of transcription in vascular smooth muscle cells. Angiotensin II is a positive regulator of plasminogen activator inhibitor-1 (PAI-1) production and also stimulates vascular smooth muscle cell proliferation. Reducing AT-R1 expression with thiazolidinediones should theoretically attenuate the overproduction of PAI-1 in patients with diabetes and reduce the potential for thrombosis. Atherosclerotic plaques are destabilized by the MMPs released by macrophages. These enzymes degrade the cross-linking collagen fibrils, promoting plaque rupture. The activation of PPAR γ in human monocyte-derived macrophage *in vitro* decreases the levels and activity of MMP-9 (the main metalloproteinase secreted by macrophage *in vitro*) (6,13,14). It is currently believed that PPAR α is involved in stimulating β -oxidation of fatty acids, mainly in the liver. In contrast, PPAR γ increases adipogenesis by stimulating adipocyte differentiation. This difference in drug action may explain the differential effects of these drugs on body

weight and abdominal fat. Thus, fenofibrate treatment decreased body weight and visceral fat, whereas rosiglitazone treatment increased body weight (15).

NUCLEAR RECEPTORS AS NOVEL DRUG TARGETS

Peroxisome proliferator-activated nuclear receptors represent novel targets for the development of therapeutic agents for the treatment of chronic diseases such as type 2 diabetes mellitus and the metabolic syndrome. However, despite the proven benefits of targeting PPARs, they display certain side effects which limit their clinical use. The thiazolidinedione class side effects include fluid retention, peripheral edema, mild anemia, weight gain and possible increased risk for congestive heart failure. The relationship between thiazolidinedione use and congestive heart failure has now been demonstrated in a number of studies. The association is felt to be the result of increased fluid retention and expansion of plasma volume by thiazolidinediones. However, the underlying mechanism for this volume expansion is not fully known (16,17,18).

PPAR γ partial agonists, PPAR α and PPAR γ dual agonists and panagonists (α , β/δ , γ PPAR activators) are currently being evaluated in clinical trials, and offer significant advantages relating to glycemic control, lipid profiles and weight gain compared with the first generation of thiazolidinedione drugs (19).

More powerful new compounds with pan- (α , β/δ , γ) PPAR activity and proven long-term safety should be highly effective in a clinical setting for patients with coexisting relevant lipid and glucose metabolism disorders.

The new generation of dual-action PPARs, the glitazars, have been developed, which target PPAR γ and PPAR α (like muraglitazar and tesaglitazar) and have potential as a treatment of global risk in patients with type 2 diabetes or metabolic syndrome. Their long-term clinical effects are still unknown. Combined treatments with PPAR γ and PPAR α agonists may potentially improve insulin resistance and alleviate atherogenic dyslipidemia. A number of glitazars (including ragaglitazar and farglitazar) have presented

problems at a late stage of clinical trial because of serious side effects. Tesaglitazar revealed side effects such as an increase in serum creatinine and a decrease in glomerular filtration rate (19). A meta-analysis that evaluated the incidence of death and major adverse cardiovascular events in diabetic patients treated with muraglitazar reported that compared with placebo or pioglitazone, muraglitazar was associated with an excess incidence of the composite endpoint of death, major adverse cardiovascular events (myocardial infarction, stroke, transient ischemic attack) and congestive heart failure. This study suggests that muraglitazar should not be approved to treat diabetes until safety is documented in a dedicated cardiovascular events trial (20). Residual safety concerns about carcinogenicity have not yet been completely resolved either.

To overcome the unfavorable actions of these agents, selective PPAR modulators (SPPARMs) are currently being developed. Recent drug discovery studies have focused on identifying new non-thiazolidinedione selective PPAR γ modulators with improved efficacy and/or safety profiles. These approaches include metaglidasen, the most advanced SPPARM. In type 2 diabetic patients, metaglidasen is expected to demonstrate antidiabetic activity while inducing fewer side effects compared to currently used thiazolidinediones. However, their role and safety profile in clinical medicine remains to be proven (19).

CLINICAL TRIALS

The DREAM (Diabetes Reduction Assessment with ramipril and rosiglitazone Medication) trial is evaluating the prevention of diabetes by rosiglitazone in individuals with impaired glucose tolerance, along with the prevention of cardiovascular events. In this trial, rosiglitazone treatment reduced incident type 2 diabetes and increased the likelihood of regression to normoglycemia in adults with impaired fasting glucose or impaired glucose tolerance (21).

The Diabetes Outcome Progression Trial (ADOPT) was a large, international, randomized, double-blind, controlled study performed to evaluate the long-term glucose-lowering efficacy of a thiazolidinedione

compared with that of metformin and glyburide. The study included 4351 subjects aged 30-75, recently diagnosed with type 2 diabetes. The patients were randomized to receive either rosiglitazone (n=1456), metformin (n=1454), or glyburide (n=1441) monotherapy. At 5 years, the cumulative incidence of treatment failure defined as confirmed hyperglycemia (fasting blood glucose 10.0 mmol/L) on consecutive testing after at least 6 weeks of treatment at the maximum-dictated or maximum-tolerated dose of the study drug was 15% with rosiglitazone, 21% with metformin, and 34% with glyburide. Rosiglitazone was associated with a greater risk of cardiovascular events than glyburide. Weight gain and peripheral edema were more common with rosiglitazone than with either metformin or glyburide. In this study, the durability of glycemic control achieved with rosiglitazone was offset by the greater risk of cardiovascular events, a result that foreshadowed widely publicized concerns regarding the safety of thiazolidinediones (22).

PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) was a large, multicenter, randomized, placebo-controlled trial performed to evaluate whether pioglitazone reduced macrovascular morbidity and mortality in high-risk individuals with type 2 diabetes. The study included 5238 type 2 diabetic patients with established cardiovascular disease. The patients were randomized to receive either pioglitazone (n=2605) or placebo (n=2633) in addition to their regular glucose-lowering regimen. At randomization, 62% of patients were taking metformin, 62% were taking a sulfonylurea alone or in combination with another agent, and >30% were taking insulin. The primary endpoint was the proportion of individuals that experienced a composite of all-cause mortality, nonfatal myocardial infarction, acute coronary syndrome, stroke, endovascular or surgical intervention in the coronary or leg arteries, and above-ankle amputation. The main secondary endpoint was a composite of all-cause mortality, nonfatal myocardial infarction, and stroke. After a mean of 34.5 months, 514 (19.7%) individuals in the pioglitazone group and 572 (21.7%) in the placebo group had at least 1 event in the primary endpoint

($P=0.095$), while 301 (11.6%) and 358 (13.6%) individuals in these groups had at least 1 event in the main secondary endpoint ($P=0.027$). In this trial, pioglitazone treatment resulted in only modest reduction in the risk of the primary composite endpoint (23).

The safety of thiazolidinediones as a class for potentially increasing the risk of acute myocardial infarction and cardiovascular death has recently garnered attention following a meta-analysis by Nissen and Wolski (18). The recent widely publicized meta-analyses suggest that rosiglitazone may increase the risk of acute myocardial infarction and cardiovascular death (24,25).

However, interim analysis of a randomized trial designed to assess cardiovascular outcomes in type 2 diabetic patients on rosiglitazone did not show an increased risk of acute myocardial infarction (26,27).

Thiazolidinediones (pioglitazone and rosiglitazone) are not associated with a higher risk of acute myocardial infarction, as recently reported by Habib *et al.* When compared with rosiglitazone, pioglitazone appeared to have a more favorable cardiovascular risk profile, resulting in fewer hospitalizations for congestive heart failure and coronary heart disease events (28).

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CONCLUSION

As we entered the new millennium, we are still facing the challenge of an explosion in the prevalence of diabetes. New approaches in the treatment of diabetes will focus on improving insulin sensitivity.

The peroxisome proliferator-activated receptors (PPARs) are promising targets for the development of new drugs for the treatment of type 2 diabetes, obesity, dyslipidemia, atherosclerosis and the metabolic syndrome. PPAR γ agonists such as thiazolidinediones improve insulin resistance and type 2 diabetes, while PPAR α agonists such as fibrates improve dyslipidemia.

The thiazolidinediones rosiglitazone and pioglitazone are currently approved for the treatment of hyperglycemia in patients with type 2 diabetes mellitus. The thiazolidinedione class side effects include fluid retention, peripheral edema, mild anemia, weight gain and possibly an increased risk of congestive heart failure.

A variety of novel therapies for type 2 diabetes are being developed, which will provide more choices for the management of the disease to both patients and diabetes care providers. Importantly, many of these treatments offer a potential to significantly improve multiple metabolic parameters.

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