History of Discovery

Fibrates, Glitazones, and Peroxisome Proliferator–Activated Receptors

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Abstract—Several decades ago, fibrates were approved for the treatment of dyslipidemia, whereas thiazolidinediones were screened in animal models to improve glucose homeostasis and were subsequently developed for the treatment of type 2 diabetes mellitus. Relatively recently, these drugs were found to act via peroxisome proliferator–activated receptors, nuclear receptors that control lipid metabolism and glucose homeostasis. In this historical perspective, we discuss the history of discovery of the peroxisome proliferator–activated receptors, from the clinical development of their agonists to the subsequent discovery of these receptors and their mechanisms of action, to finally evoke possibilities of targeted pharmacology for future development of selective peroxisome proliferator–activated receptor modulators. 

(Key Words: atherosclerosis ■ pharmacology ■ glucose homeostasis ■ lipid metabolism ■ nuclear receptors)

Fibrates and Glitazones: Classic Pharmacology and Clinical Use

Discovery of Fibrates

The first fibrates were synthesized in the mid-1950s. Their clinical development came from the observation, in 1953, of researchers in France that, among 80 synthesized structures derived from dehydrocholic acid, phenylethyl acetic acid, and certain other disubstituted acetic acids exhibited hypcholesterolemic properties in rats and humans.3,12 Several years later, Imperial Chemical Industries laboratories in England screened several branched-chain fatty acids initially developed as plant hormone analogues and found that several oxyisobutyric acid derivatives decreased total lipid and cholesterol concentrations in rat plasma and liver. The most effective compound with minimal toxicity, discovered by Thorp and Waring in 1962, was ethyl-α-4-chlorophenoxyisobutyrate, which was called clofibrate. This compound was previously described in 1947 by 2 Italian chemists, Galimberti and Defranceschi, and evoked in 1956 by French researchers, Julia et al, working on plant growth–regulating substances. Clofibrate decreased lipids in animal models.3,13 Its mode of action was not known; however, initially, the hypolipidemic effect of ethyl-α-4-chlorophenoxyisobutyrate was attributed to seasonal variations in adrenal and thyroid function and the administration of androsterone in rats and monkeys potentiated the hypcholesterolemic effect of ethyl-α-4-chlorophenoxyisobutyrate.8 Subsequently, several clinical studies were performed on clofibrate as monotherapy or in combination with androsterone. These trials showed that clofibrate decreases lipid levels in hypercholesterolemic patients, mainly as the result of a reduction in the very-low-density lipoprotein, and less in the low-density lipoprotein fraction; that it was well tolerated in long-term treatment; and that the coadministration of androsterone was not necessary for its hypolipidemic effect.7,8 However, the mode of action of clofibrate was still unclear, and 30 years of intensive research was needed to discover it. Researchers were worried by the fact that long-term treatment with clofibrate induces hepatomegaly in rats as the result of unexplained proliferation of hepatic cytoplasmic inclusion bodies called peroxisomes.9 Nevertheless, clofibrate was approved in the United States in 1967 for the treatment of hyperlipidemias. Further studies suggested that the peroxisome proliferation and the hypolipidemic properties of clofibrate were independent, but the mechanism was not known. Several years later, 2 other hypolipidemic compounds, Wy-14653 and tibric acid, were more potent than clofibrate in increasing peroxisome proliferation in the liver specimens of rats and mice, despite being structurally different from clofibrate and the other oxyisobutyric acid derivatives.12

Disturbed by concerns about the clofibrate-induced hepatomegaly in murine species, Thorp and colleagues tried to modify the clofibrate structure to identify more potent hypolipidemic fibrate drugs with minimum toxicity. To do so, they used a test measuring the displacement of albumin-bound L-thyroxin because they thought that clofibrate exhibited indirect hypcholesterolemic activities because of its capacity to bind to albumin and displace albumin-bound proteins such as L-thyroxin. Two analogs were selected: methyl-
clofenapate and clobuzarit. Despite higher-potency and lipid-lowering activity when compared with clofibrate, methylclofenapate was withdrawn from clinical studies not only because of the induction of strong hepatomegaly and peroxisome proliferation in rats, but also because of the occurrence of hepatic carcinomas in rats that, at that time, were thought to be prognostic for humans. Clobuzarit was less hypolipidemic than clofibrate; however, it induced a significant decrease in plasma fibrinogen levels and was oriented toward the treatment of rheumatoid arthritis as the result of its anti-inflammatory properties.

Meanwhile, several pharmaceutical companies performed intensive research to improve the pharmacological and pharmacokinetic activities of clofibrate. Many modifications were tested: the phenoxy-2-methyl-2-propionic acid chain of clofibrate was preserved, and the Cl atom was substituted by different hydrophobic groups. However, none of the obtained phenylketone molecules were interesting hypolipidemic drugs, except for the benzoyl derivative with a Cl atom in position 4, which was called procetofen. Thus, procetofen was synthesized in 1974 and was introduced in clinical practice in France the same year. Procetofen, which significantly decreased plasma lipid concentrations in hyperlipidemic patients,13 was later called fenofibrate to comply with World Health Organization nomenclature guidelines. Fenofibrate was demonstrated to exhibit improved pharmacokinetic and pharmacological properties when compared with clofibrate.

Other fibrates were also introduced in the late 1970s and early 1980s, such as gemfibrozil in the United States and bezafibrate and cipofibrate in Europe. However, at that time, the clinical use of fibrates was limited by the demonstration that clofibrate and other hypolipidemic drugs (eg, nafenopin, Wy-14643, and tibric acid)14 induced hepatic carcinogenesis in rats and mice, suggesting a potential deleterious effect of these drugs in humans. Fortunately, in 1983, it was shown that humans and nonhuman primates seemed to be resistant to this peroxisome proliferative effect15; further epidemiological studies16,17 in humans confirmed the absence of increased risk of cancer in patients treated with fibrates, allowing these drugs to be safely used in the clinic. Consequently, a renewed interest in fibrates appeared in the 1990s when the mode of action of fibrates became understood and the results of several clinical trials, such as the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) study,18 which demonstrated the efficacy of gemfibrozil on glucose levels and to improve tissue sensitivity in rodent models and in patients with type 2 diabetes, to be orally active, and to be more selective and more potent (1 mg/kg) than ciglitazone (150 mg/kg). In parallel, Takeda laboratories in Japan developed another TZD compound, pioglitazone (AD-4833),30 which also ameliorates glucose and lipid profiles in patients with type 2 diabetes. Rosiglitazone and pioglitazone were approved in the United States in 1999 for the treatment of type 2 diabetes; today, they represent the only 2 glitazones used in the treatment of type 2 diabetes.

### Discovery of the Glitazones

Thiazolidinediones (TZDs), also termed glitazones, are widely prescribed in the treatment of type 2 diabetes as monotherapy or in combination with sulfonylureas, metformin, dipeptidyl peptidase-4 inhibitors, and insulin. However, their discovery, in the early 1980s, was quite surprising when Japanese researchers tried to synthesize more potent fibrate hypolipidemic drugs. Indeed, in 1975, Takeda laboratories in Japan synthesized 71 analogues of clofibrate (ie, alkanoic acids containing a biphenyl ether moiety) and tested them for hypolipidemic properties. Interestingly, some of these compounds showed both hypolipidemic and hypoglycemic effects in diabetic mice.21,22 Thus, extensive structure-activity relationship studies23,24 led to the discovery of ADD-3878, also called ciglitazone, which was shown to normalize hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in animal models of type 2 diabetes without provoking hypoglycemia.25 Consecutively, another glitazone, troglitazone (CS-045), discovered by Sankyo Company in 1988,26 decreased insulin resistance by both increasing insulin-stimulated glucose utilization and reducing hepatic glucose production. Troglitazone was the first TZD approved for clinical use in the United States (in 1997); unfortunately, it was subsequently withdrawn worldwide because of liver toxicity.27

Meanwhile, an intensive insulin resistance targeted drug research program had been initiated by SmithKline in the United Kingdom to develop insulin sensitizers more potent than ciglitazone. BRL-49653, synthesized in 1988 and later known as rosiglitazone, was shown to normalize blood glucose levels and to improve tissue sensitivity in rodent models and in patients with type 2 diabetes, to be orally active, and to be more selective and more potent (1 mg/kg) than ciglitazone (150 mg/kg).28,29 In parallel, Takeda laboratories in Japan developed another TZD compound, pioglitazone (AD-4833),30 which also ameliorates glucose and lipid profiles in patients with type 2 diabetes. Rosiglitazone and pioglitazone were approved in the United States in 1999 for the treatment of type 2 diabetes; today, they represent the only 2 glitazones used in the treatment of type 2 diabetes.

### Fibrates, Glitazones, and Peroxisome Proliferator–Activated Receptors: From Classic to Molecular Pharmacology

**Peroxisome Proliferator–Activated Receptor α and Fibrate Action**

In the mid-1980s, Reddy et al31 showed that fibrates increase the transcription of peroxisomal fatty acid β-oxidation genes in rat liver specimens, whereas studies in Leuven, Belgium, at the end of the 1980s showed that fibrates regulate the expression of genes involved in lipoprotein metabolism.32 The triggering event in the understanding of fibrate action occurred in 1990 when 2 researchers from the Central Toxicology Laboratories of Imperial Chemical Industries, Issmann and Green,33 identified a novel member of the steroid hormone receptor superfamily of ligand-activated transcription factors. The receptor was structurally related, but clearly different, from steroid hormone receptors; could
be activated by a wide range of molecules, including several fatty acids and the pharmacological class of fibrates; was thought to mediate the peroxisome proliferation response\textsuperscript{33}; and was, therefore, called peroxisome proliferator–activated receptor (PPAR), later PPAR\(\alpha\) (NR1C1). This receptor was expressed at a high level in the liver, kidney, and heart. Further studies\textsuperscript{34,35} showed that PPAR activation increases the transcription of specific PPAR target genes after heterodimerization with the retinoid X receptor and binding of the complex to specific transcriptional regulatory elements called peroxisome proliferator response elements. It was hypothesized that the hypolipidemic effects of fibrates were mediated through PPAR\(\alpha\);\textsuperscript{36} which was proven by using knockout mouse models that allow the study of the in vivo role of this receptor. Thus, using the PPAR\(\alpha\) knockout mouse,\textsuperscript{37} it was shown that fibrates decrease plasma lipid levels and induce hepatomegaly and hepatic peroxisome proliferation in a PPAR\(\alpha\)-dependent manner. Moreover, species differences in PPAR\(\alpha\) function, particularly between murine species and humans, were found at the level of PPAR\(\alpha\) expression, ligand activation, and biological responses, especially with respect to its role in peroxisome proliferation in rats and mice, but not humans.\textsuperscript{38}

By using molecular biology and functional genomic technologies, target genes of PPARs were identified, with roles not only in lipid homeostasis but also in other pathways. Thus, in humans, the increase in serum high-density lipoprotein cholesterol (HDL-C) concentrations is related to the gene activation of 2 major HDL apolipoproteins, apolipoprotein A-I and apolipoprotein A-II, by PPAR\(\alpha\).\textsuperscript{39} Moreover, the triglycerides (TG)-lowering action of fibrates, related to decreased synthesis and increased catabolism of very-low-density lipoprotein, was associated with the inhibition of apolipoprotein C-III expression, a well-known inhibitor of lipoprotein lipase, and the induction of lipoprotein lipase and apolipoprotein A-V expression. In addition to its major role in lipid homeostasis, PPAR\(\alpha\) has exhibited additional pleiotropic effects on endothelial dysfunction, myocardial ischemic injury, and immune-inflammatory responses. At that time, PPAR\(\alpha\) was expressed in the major cell types of the atherosclerotic plaque, including macrophages, smooth muscle cells, endothelial cells, and lymphocytes; its activation resulted in direct antiatherogenic effects in the artery wall.\textsuperscript{40} PPAR\(\alpha\) activation reduces the recruitment and adhesion of mononuclear cells to the endothelium, decreases atherosclerotic plaque inflammation and proliferation of smooth muscle cells, and facilitates cholesterol efflux by increasing the expression of the transporter scavenger receptor-B1 and ATP-binding cassette transporter A-1 in macrophages. Thus, these molecular actions of PPAR\(\alpha\) increased our understanding of the hypolipemic effects of fibrates and suggested an interesting potential of these drugs in the control of cardiovascular disease and its risk factors.

PPAR\(\gamma\) and Glitazone Action

After the discovery of PPAR\(\alpha\) in 1990, 2 other genes belonging to the same family, PPAR\(\beta/\gamma\) (NR1C2) and PPAR\(\gamma\) (NR1C3), were cloned in 1992.\textsuperscript{36} PPAR\(\gamma\) is a nuclear receptor that regulates gene transcription after activation by small lipophilic fatty acid derivatives or oxidized lipid components of oxidized low-density lipoprotein (9-hydroxy and 13-hydroxy octadecadienoic acids). In 1994, PPAR\(\gamma\) was shown to be a major adipogenic transcription factor\textsuperscript{41,42}; in 1995, PPAR\(\gamma\) was identified as the target of the TZDs.\textsuperscript{43} Therefore, PPAR\(\gamma\) was suggested to mediate the antiatherogenic actions of TZDs. Even though the molecular mechanisms by which TZDs exert hypoglycemic and insulin-sensitizing effects are not totally understood, adipose tissue seems to be the major organ implicated. Indeed, PPAR\(\gamma\) promotes adipocyte differentiation; stimulates fatty acid storage in adipocytes via the activation of genes, such as lipoprotein lipase, fatty acid transport protein, CD36, and acyl-coA synthetase; and decreases free fatty acid secretion, resulting in enhanced adipocyte insulin signaling. This effect can explain the body weight gain induced by TZD administration, a major drawback in the use of these drugs in the treatment of type 2 diabetes. Consequently, plasma free fatty acid levels are decreased, thus improving lipotoxicity and insulin sensitivity in liver and skeletal muscle. These insulin-sensitizing effects result in long-term glycemic control in patients with type 2 diabetes who receive treatment with TZDs, as shown A Diabetes Outcome Progression Trial (ADOPT).\textsuperscript{44} PPAR\(\gamma\) activation also decreases obesity-induced inflammation and insulin resistance by regulating the expression of cytokines (tumor necrosis factor) and adipokines (adiponectin and resistin) in adipose tissue. Moreover, PPAR\(\gamma\) is expressed in atherosclerotic plaques; also, the fact that TZDs inhibit inflammatory cytokine secretion by activated macrophages increased their interest as potential antiatherogenic drugs. The PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive Study) has indicated the protective effects of pioglitazone against macrovascular diseases in patients with type 2 diabetes.\textsuperscript{45}

PPAR\(\beta/\gamma\)/PPAR\(\delta\): A Target for Novel Drugs

PPAR\(\delta\), also known as nuclear hormone receptor 1 (NUC1) or PPAR\(\beta\), is activated by saturated and polysaturated fatty acids and eicosanoids. Synthetic PPAR\(\beta/\gamma\)/PPAR\(\delta\) ligands have been developed, such as the phenoxyacetic derivatives GW501516 and GW0742, optimized from a library of hydrophobic carboxylates or L165461 obtained from an in silico approach.\textsuperscript{46,47} Although there are no drugs activating PPAR\(\beta/\gamma\)/PPAR\(\delta\) that are approved for clinical treatment, new synthetic PPAR\(\beta/\gamma\)/PPAR\(\delta\) agonists are in development for the treatment of dyslipidemia, obesity, and/or insulin resistance, such as MBX-8025, CER-002, and KD3010. Activation of PPAR\(\beta/\gamma\)/PPAR\(\delta\) reduces adiposity and improves glucose metabolism and insulin sensitivity in animal models of obesity and insulin resistance, an effect likely due to the capacity of PPAR\(\beta/\gamma\)/PPAR\(\delta\) to enhance fatty acid transport and oxidation.\textsuperscript{48,49} Moreover, PPAR\(\beta/\gamma\)/PPAR\(\delta\) activation improves dyslipidemia by increasing the HDL level,\textsuperscript{50} a beneficial effect being investigated in humans. PPAR\(\beta/\gamma\)/PPAR\(\delta\) is also implicated in the adaptive metabolic response of skeletal muscle to exercise endurance by increasing oxidative muscle fibers.\textsuperscript{51,52} Furthermore, PPAR\(\beta\) or PPAR\(\gamma\) is expressed in vascular cells, including endothelial cells, smooth muscle cells, and macrophages; PPAR\(\beta/\gamma\)/PPAR\(\delta\) activation has direct beneficial effects on vascular homeostasis by protecting endothelial
cells from inflammation and apoptosis, decreasing the proliferation of smooth muscle cells (SMCs), decreasing macrophage inflammation, and promoting angiogenesis. These effects confer a promising potential of PPARβ/PPARδ agonists for the treatment of the metabolic syndrome in humans.

Future PPAR Agonists: From Basic Molecular to Target-Oriented Pharmacology

PPAR Coagonists

The discovery of the role of the PPARs in the regulation of lipid and glucose metabolism led the pharmaceutical industry to develop PPAR coagonists. Because many patients with insulin resistance also exhibit dyslipidemia, it was theoretically judicious to create dual PPARα/PPARγ agonists to treat these patients. Indeed, these dual PPARα/PPARγ agonists, also named glitazars, combine the insulin-sensitizing effects of PPARγ activation with the lipid-lowering effects of PPARα agonists, lowering triglycerides, increasing the HDL level and insulin sensitivity, and reducing cardiovascular risk. In animal models of insulin resistance and dyslipidemia, these compounds were promising. However, in humans, despite an improved clinical efficacy on glucose and lipid metabolism, several dual PPARα/PPARγ agonists were withdrawn in late-stage clinical development, mostly because of safety concerns. For example, ragaglitazar development was discontinued in 2004 as the result of the induction of anemia and urothelial cancer. The development of muraglitazar and tesaglitazar was discontinued in phase 3 clinical trials in 2006 because of edema and major adverse cardiovascular events (ie, myocardial infarction, stroke, and heart failure) for muraglitazar and elevated serum creatinine levels and a decreased glomerular filtration rate for tesaglitazar. These discouraging adverse effects consequently raised questions about the therapeutic potential of such drugs. However, the recent results with aleglitazar, showing improved safety, higher efficacy, and encouraging, albeit still short-term, cardiovascular effects, give real hope for the future of glitazars.

Dual PPARγ/PPARδ and PPARα/PPARδ agonists are also under investigation as hypolipidemic, hypoglycemic, and antiatherogenic agents. The dual PPARγ/PPARδ agonist, (R)-3-{2-ethyl-4-[3-(4-ethyl-2-pyridin-2-yl-phenoxy)-butoxy]-phenyl}propionic acid lowered plasma glucose levels and induced less weight gain than rosiglitazone in Zucker diabetic fatty rats. As for the dual PPARα/PPARδ agonists, T913659 has shown beneficial lipid effects in primates and GFT505 has shown promising results in a phase 2 clinical trial in the management of dyslipidemia associated with abdominal obesity. However, further clinical investigation is needed to explore their potential clinical use in diabetes-associated macrovascular and microvascular complications.

Finally, the development of agonists combining the effects of the 3 PPARs is under investigation. Interestingly, bezafibrate, an older-generation fibrate, is a weak pan PPARα/PPARγ/PPARδ agonist. Bezafibrate increases the HDL-C level, decreases TG levels, improves insulin sensitivity, and decreases the long-term rate of progression of coronary artery disease. Consequently, novel PPAR pan agonists (eg, PLX-204, GW-625019, and GW-677954) are currently investigated as clinical agents in the treatment of type 2 diabetes and its cardiovascular complications.

Selective PPAR Modulators

Resulting from a better understanding of the molecular actions of nuclear receptors, the concept of selective PPAR modulators, in analogy to selective estrogen receptor modulators, emerged recently as a means to optimize the therapeutic potential. This new molecular target–based strategy aims to synthesize a PPAR agonist with a target-oriented therapeutic profile, maintaining the desired therapeutic benefit and minimizing the adverse effects of the first-generation PPAR agonists. At the molecular level, each PPAR ligand induces a specific change in PPAR conformation, resulting in the differential recruitment of cofactors and gene-specific transcriptional regulation. Thus, in addition to a panel of common genes regulated in a similar manner by all agonists, each agonist also induces its proper profile of genes, resulting in specific biological effects. The validity of the concept is clinically supported by observations that fenofibrate and gemfibrozil, which both increase HDL-C levels, have distinct effects on human apolipoprotein A-I concentrations. Thus, new compounds were created with differential gene-regulating properties. This concept of selective PPAR modulators is particularly relevant for PPARγ agonists. Indeed, because rosiglitazone and pioglitazone display well-documented adverse effects, such as body weight gain, increased risk for bone fractures, and edema, the idea is to develop compounds retaining the beneficial effects of these full PPARγ agonists on glucose metabolism without the adverse effects. Several TZD-like and non–TZD-like PPARγ partial agonists or selective PPAR modulators have been synthesized, and some of them have entered clinical studies (eg, halofenate or metaglidasen, DRF-2593 [balaglitazone], or MCC555 [netoglitazone]).

Conclusions

Fibrates and TZDs were developed decades ago for the treatment of dyslipidemia and type 2 diabetes. However, since 1990, their mechanism, through the discovery of the PPARs, began to be elucidated. Since that time, PPARs have been shown to be implicated in many processes, such as lipid and glucose metabolism, inflammation, or cardiovascular functions, thus presenting PPARs as interesting pharmacological targets. In addition, the use of PPAR ligands enabled advancements regarding the physiological roles of PPARs. Although none of the new synthetic PPAR agonists, PPAR coagonists, or selective PPAR modulators have replaced the first-generation fibrates and TZDs, they constitute a panel of promising drugs. We are sure that PPARs have not revealed all their secrets and will continue to interest researchers who are trying to understand their physiological roles and their potential as targets for drugs to prevent cardiometabolic disorders.

Disclosures

Bart Staels has participated in educational symposia organized by Solvay, Takeda and GSK and is a consultant of Genfit.
References


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