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Review

Peroxisome Proliferator-Activated Receptor (PPAR)-α: A Pharmacological Target with a Promising Future

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Peroxisome proliferator-activated receptor (PPAR)-α is a ligand-activated transcriptional factor that belongs to the family of nuclear receptors. PPAR-α regulates the expression of genes involved in fatty acid β-oxidation and is a major regulator of energy homeostasis. Fibrates are PPAR-α agonists and have been used to treat dyslipidemia for several decades because of their triglyceride (TG) lowering and high-density lipoprotein cholesterol (HDL-C) elevating effects. More recent research has demonstrated anti-inflammatory and anti-thrombotic actions of PPAR-α agonists in the vessel wall as well. Thus, PPAR-α agonists decrease the progression of atherosclerosis by modulating metabolic risk factors and by their anti-inflammatory actions on the level of the vascular wall. This is confirmed by several clinical studies, in which fibrates have shown to reduce atherosclerotic plaque formation and the event rate of coronary heart disease (CHD), especially in patients suffering from metabolic syndrome (MS). MS is characterized by a group of metabolic risk factors that include obesity, raised blood pressure, dyslipidemia, insulin resistance or glucose intolerance, and a prothrombotic state, and its incidence in the Western world is rising to epidemic proportions. This review paper will focus on the functions of PPAR-α in fatty acid β-oxidation, lipid metabolism, and vascular inflammation. Furthermore, PPAR-α genetics, the clinical use of PPAR-α activators and their future perspective will be discussed.

KEY WORDS: atherosclerosis; fatty acid oxidation; fibrates; inflammation; lipoproteins; metabolic syndrome; peroxisome proliferator-activated receptor (PPAR)-α; PPAR-α L162V polymorphism.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death and disability for both men and women in the Western world (1). The relationship between CVD and disorders of cholesterol metabolism is well-established (2). Several strategies to lower high blood cholesterol levels have been developed. The statin class of drugs has been the primary therapeutic agent and has shown in both primary (3) and secondary (4) clinical trials to dramatically reduce the event rate of coronary heart disease (CHD). However, not all patients with dyslipidemias benefit from statin treatment and some conditions such as low high-density lipoprotein cholesterol (HDL-C) levels, often combined with elevated triglyceride (TG) levels, remain refractory to treatment. This explains a continuous search for novel therapeutic agents.

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ABBREVIATIONS: ABCA1, ATP-binding cassette transporter A1; ACC, acetyl-coenzyme A carboxylase; ACO, acyl-coenzyme A oxidase; ACS, acyl-coenzyme A synthetase; AF, activation function; Apo, apolipoprotein; BECAIT, Bezafibrate Coronary Atherosclerosis Intervention Trial; BIP, Bezafibrate Prevention Study; CARDS, Collaborative Atorvastatin Diabetes Study; CHD, coronary heart disease; COX, cyclo-oxygenase; CPT I, carnitine palmitoyl transferase I; CPT II, carnitine palmitoyl transferase II; CRP, C-reactive protein; CT, carnitine: acylcarnitine translocase; CVD, cardiovascular disease; DAIS, Diabetes Atherosclerosis Intervention Studies; DBD, DNA binding domain; DM, diabetes mellitus; DR-1, direct repeat spaced by one nucleotide; FABP, fatty acid binding protein; FAS, fatty acid synthase; FATP, fatty acid transport protein; FFA, free fatty acid; FIELD, Fenofibrate Intervention and Event Lowering in Diabetes; HD, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; HRE, hormone response element; IL, interleukin; IR, insulin resistance; LBD, ligand binding domain; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; LTB_4, leukotriene β_4; LOCAT, Lopid Coronary Angiography Trial; LPL, lipoprotein lipase; LXR-α, liver-X-receptor-α; MAP, mitogen activated protein; MCAD, medium chain acyl-coenzyme A dehydrogenase; MI, myocardial infarction; MS, metabolic syndrome; NF-κβ, nuclear factor-kappa bèta; NSAIDS, nonsteroidal anti-inflammatory drugs; P450, cytochrome P450 fatty acid ω-hydroxylase; PLTP, phospholipid transfer protein; PPAR-α, peroxisome proliferator-activated receptor-α; PPAR-γ, peroxisome proliferator-activated receptor-γ; PPAR-δ, peroxisome proliferator-activated receptor-δ; PPRE, peroxisome proliferator response element; RCT, reverse cholesterol transport; RXR-α, retinoid-X-receptor-α; SMC, smooth muscle cell; TC, total cholesterol; TF, tissue factor; TG, triglyceride; TNF-α, tumor necrosis factor-α; TRL, triglyceride rich lipoprotein; VA-HIT, Veterans Affair HDL Intervention Trial; VCAM-1, vascular adhesion molecule-1; VLDL, very low density lipoprotein; WT, wild type.

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Orphan nuclear receptors were discovered in the early 1990s. These transcriptional factors regulate the expression of a large number of genes that are involved in lipid and carbohydrate metabolism (5). Over the past years an extensive body of research has underlined their potential as therapeutic targets for the treatment of dyslipidemias and atherosclerosis. This review will focus on the peroxisome proliferator-activated receptor (PPAR)-α, an orphan nuclear receptor involved in the regulation of fatty acid metabolism.

ORPHAN NUCLEAR RECEPTORS

Nuclear receptors function as ligand-activated transcription factors that regulate the expression by binding directly to the DNA of their target genes. Each receptor recognizes a specific DNA sequence, called a hormone response element (HRE), which is usually located in the promoter region of the target gene. Nuclear receptors mediate the actions of numerous hormones and polypeptides and affect processes as diverse as development, homeostasis and energy metabolism (5). One part of the nuclear receptor superfamily includes the "classic" nuclear steroid receptors. These are the receptors for the hormones estrogen, progesterone, androgens, and the corticoid hormones. Their ligands, and the pathways they regulate, have been investigated extensively and their mechanisms of action are well-understood (6).

In addition to these well-known nuclear receptors, Evans speculated that more hormonal systems could be involved in complicated processes like homeostasis and development, based on the discovery of a great number of receptor-related molecules in a wide range of species (7). After the finding that the classic nuclear receptors share extensive homology at the DNA sequence level, well-conserved receptor fragments were used as probes to screen cDNA libraries to identify new receptors. At first, the cDNAs of two receptor-like genes were cloned (8). Subsequently, more receptors were isolated, and because their ligands and functions were unknown, they were initially called orphan nuclear receptors. They now form the second part of the nuclear receptor superfamily. Currently, a total of 48 members of this superfamily have been identified in the human genome (9,10).

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-α

In 1990, Issemann *et al.* (11) discovered that peroxisome proliferators, a heterogeneous group of compounds, activated one of these orphan nuclear receptors that includes the hypolipidemic drug clofibrate. Their name was based on the observation that these agents induce proliferation of the cell organelle peroxisomes in rodents (12). Hence, the orphan nuclear receptor was named "peroxisome proliferator-activated receptor." Human PPAR-α was cloned in 1993 (13). A couple of years later, the cDNA of the other members of this nuclear receptor subfamily, PPAR-γ and PPAR-δ, was cloned (14,15). Although none of the members of the PPAR family actually induce peroxisome proliferation in humans, the name has not been changed.

MECHANISM OF ACTION

PPAR-α is a ligand-activated transcriptional factor that binds to a DNA sequence known as a peroxisome proliferator response element (PPRE), which is located in the promoter region of the target gene (16) (Fig. 1). A PPRE usually consists of two direct repeats of six nucleotides, spaced by one or two nucleotides and are often referred to as DR1 or DR2 respectively (17). Upon activation by biologic or synthetic ligands, PPAR-α heterodimerizes with the retinoid X receptor (RXR)-α and undergoes conformational changes, which enables binding of the PPAR-α-RXR-α vehicle to the PPRE. Ligands of PPAR-α and RXR-α alone can induce transcription, but when both receptors are activated by their ligands at the same time, they synergistically enhance the transcription of genes (18,19). In addition to ligand-dependent activation, PPAR-α can also be regulated by phosphorylation of two mitogen-activated protein (MAP) kinase sites located in the modulator region of the receptor (20). A number of hormones, for instance insulin, can modulate PPAR-α activity through this pathway (21).

STRUCTURE OF PPAR-α

PPAR-α has a comparable structural organization as other nuclear receptors. The receptor consists of four functional modules (Fig. 2). These are the modulator region, the DNA binding domain (DBD), the hinge region, and the ligand binding domain (LBD). The modulator region contains a ligand-independent activation function (AF)-1 located at the amino-terminal end, which can be activated by phosphorylation through the MAP kinase sites. The DBD is composed of two zinc fingers and binds to the PPRE. The function of the hinge region is not well understood, but its structure is very flexible and may be crucial for efficient binding of the DBD to the target sequence. The LBD mediates ligand binding and dimerization with RXR-α. Ligand-dependent transactivation is dependent on the presence of the AF-2, positioned at the carboxy terminus of PPAR-α LBD (5).

TISSUE EXPRESSION, LIGANDS, AND TARGET GENES

PPAR-α is mainly expressed in tissues with elevated mitochondrial and peroxisomal fatty acid β-oxidation rates, such as liver, heart muscle, kidney, skeletal muscle, and brown fat (22,23). PPAR-α is also present in cells of the arterial wall, in monocytes/macrophages (24), smooth muscle cells (25), and endothelial cells (26).

After the identification of PPAR-α as the receptor for

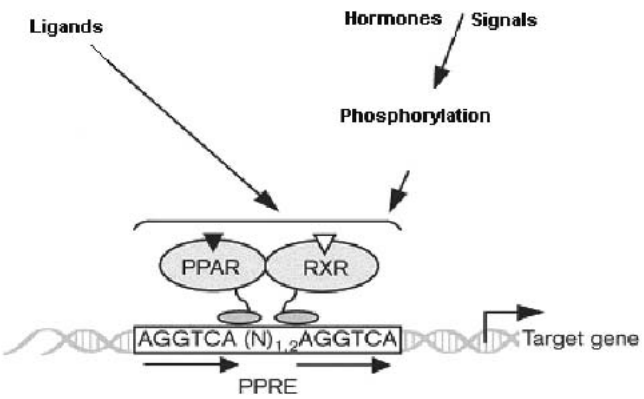

Fig. 1. Mechanism of transcription regulation by PPAR-α through a PPRE (adapted from Torra *et al.*, 2001 (16)).

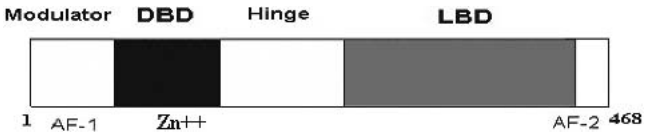

Fig. 2. Schematic structure of PPAR-α.

clofibrate, numerous groups searched for alternative activators. It was discovered that saturated and unsaturated fatty acids are the primary *natural* PPAR-α ligands (27). Moreover, PPAR-α activity was shown to be induced by eicosanoids (28) and leukotriene β_4 (29). In addition to clofibrate, other *synthetic* compounds can also activate PPAR-α, including carbaprostacyclin (30), nonsteroidal anti-inflammatory drugs (31), pirinixic acid (also known as WY-14643), phthalate ester plasticizers, and the second-generation fibrates (e.g. fenofibrate, bezafibrate, and gemfibrozil) (32). Finally, PPAR-α is induced by glucocorticoids in response to stress and follows a diurnal rhythm (33). The search for new compounds that specifically target PPAR-α are being sought out but little has been published in the public domain at the time this article was written.

PPAR-α plays a crucial role in intracellular lipid metabolism. It regulates the expression of proteins involved in the transport and β-oxidation of free fatty acids (FFAs), predominantly in the liver (Fig. 3). Specifically, PPAR-α upregulates fatty acid transport protein (FATP), which facilitates the uptake of long chain fatty acids by the liver. After passage across the plasma membrane, the fatty acids are either esterified and activated by acyl-coA synthetase (ACS) to acyl-coA derivates or bound by fatty acid binding protein (FABP). In this way, escape of free fatty acids from the cell is prevented. Both the ACS- and the FABP gene contain a PPRE in their promoters. PPAR-α promotes β-oxidation of the activated acyl-CoA esters by inducing the enzymes medium chain acyl-coenzyme A dehydrogenase (MCAD), acyl-coA oxidase (ACO), and cytochrome P450 fatty acid ω-hydroxylase. These are the key enzymes involved in β-oxidation in mitochondria, peroxisomes and microsomes, respectively (34). Furthermore, the enzyme carnitine palmitoyl transferase I (CPTI) is up regulated by PPAR-α through a functional PPRE in the promoter region of the gene (35). This enzyme catalyzes the rate-limiting step in the translocation of activated acyl-CoA esters across the mitochondrial membranes and is therefore crucial for mitochondrial β-oxidation. The finding that all of these enzymes are induced even at very low concentrations of PPAR-α agonists, underlines their importance in energy homeostasis and fatty acid metabolism. Also, PPAR-α reduces *de novo* fatty acid synthesis by blocking enzymes like acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (34). These data are supported by a study in PPAR-α (–/–) mice. PPAR-α knockout mice failed to show increased transcription of the enzymes involved in fatty acid transport and β-oxidation after stimulation with fibrates and developed intrahepatic accumulation of lipid droplets after two weeks of rodent chow diet (36).

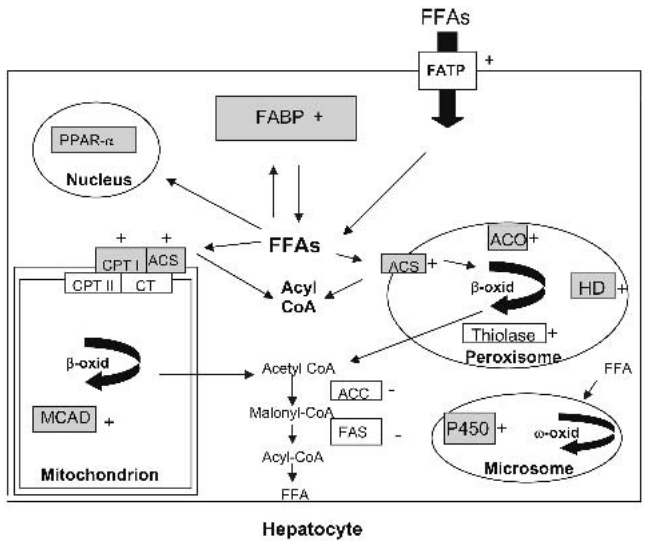

Fig. 3. Summary of the different genes regulated by PPAR-α and their roles in intracellular lipid metabolism. The genes encoding the proteins indicated in a gray box contain a functional PPRE. The proteins in a white box are regulated by PPAR-α, but no functional PPRE has been identified. The + or – sign indicates whether gene expression is stimulated or inhibited (adapted from Schoonjans *et al.*, 1996 (33)).

In summary, PPAR-α functions as a fatty acid sensor and by activating its target genes, it is a major regulator of fatty acid metabolism and energy homeostasis.

PPAR-α EFFECTS ON LIPOPROTEIN METABOLISM

PPAR-α agonists have a pronounced effect on lipoprotein metabolism, both inside as outside the liver. Especially the metabolism of triglyceride-rich lipoproteins (TRLs) is strongly affected (37). Because of the induction of enzymes involved in the β-oxidation pathway in the liver, FFA metabolism is shifted from TG synthesis to catabolism. Consequently, the secretion of very low density lipoproteins (VLDL) particles by the liver is strongly reduced (34). Additionally, PPAR-α activators decrease plasma TRLs levels by increasing the activity of the enzyme lipoprotein lipase (LPL) (38). LPL is the key enzyme in the hydrolysis of triglycerides in TRLs and also mediates the uptake of atherogenic TRL remnants by the liver. LPL activity is up-regulated in a direct and indirect way. Directly, PPAR-α agonists increase transcriptional activity of the LPL gene promoter, which contains a PPRE (39). Indirectly, PPAR-α ligands reduce the levels of apolipoprotein (apo) C-III, a natural inhibitor of LPL activity (40). Through these combined actions, TRL plasma levels are markedly reduced by PPAR-α activators.

An additional beneficial effect of the decrease in plasma TG levels, is the increase of low-density lipoprotein (LDL) particle size. The larger, more buoyant LDL particles formed by the TG-lowering actions of PPAR-α agonists, are less susceptible to oxidation and therefore less atherogenic than small, dense LDL particles (41,42).

PPAR-α activators also mediate favorable actions on high-density lipoprotein (HDL) metabolism and, therefore, affect the anti-atherogenic reverse cholesterol transport (RCT). RCT is the process in which HDL particles mediate the uptake of cholesterol from lipid laden peripheral cells (including those in the vascular wall) with subsequent delivery back to the liver, where cholesterol is excreted into the bile as bile salts (43). PPAR-α activators modulate a number of steps in this process. First of all, PPAR-α agonists upregulate the synthesis of apo A-I and A-II in the liver (44,45).

Apo A-I and apo A-II are the two major apolipoproteins of the HDL fraction and they participate in HDL particle formation. Furthermore, PPAR-α agonists promote HDL maturation through the enhanced hydrolysis of TRLs. Increased hydrolysis of TRLs leads to an increased synthesis of constituents that contribute to the HDL pool (46). In a recent study in mice fibrates were also shown to increase the expression and activity of phospholipid transfer protein (PLTP), an important modulator in HDL formation (47). Finally, PPAR-α agonists up-regulate the expression of the ATP-binding cassette transporter A1 (ABCA1) in macrophages. This transmembrane protein is responsible for cellular cholesterol efflux, which forms the first step in RCT. PPAR-α agonists induce ABCA1 expression by an indirect mechanism through the nuclear liver X receptor (LXR)-α, which contains a PPRE in its promoter (48).

The role of PPAR-α in bile acid synthesis is still controversial. PPAR-α induces the hepatic expression of LXR-α (49). In rats, LXR-α up-regulates the expression of cholesterol 7α-hydroxylase (CYP7A), a protein that promotes conversion of cholesterol to bile acids in the liver (50). Cheema *et al.* found a modest induction of the human CYP7A1 promoter by PPAR-α agonists (51). By contrast, fibrates were shown to reduce CYP7A activity in both rodents and humans in other studies (52,53). The major actions of PPAR-α on lipid homeostasis and RCT are summarized in Fig. 4.

PPAR-α EFFECTS ON ATHEROGENESIS

Atherosclerosis is a chronic complex process that is thought to be initiated by local inflammatory responses as a prelude to the accumulation of lipids in the vascular wall (54). PPAR-α is expressed in a number of cells in the vessel wall (24–26). The first indication that PPAR-α modulates vascular inflammation came from a study in PPAR-α (–/–) mice, which showed a prolonged inflammatory response (29). Additionally, fibrates were shown to reduce atherosclerotic plaque formation independent of changes in plasma lipid levels in both animals (55) and humans (56). Numerous groups investigated the anti-inflammatory properties of PPAR-α. In endothelial cells, Marx *et al.* showed that PPAR-α activators inhibited cytokine-induced expression of vascular adhesion molecule-1 (VCAM-1), a protein that stimulates the uptake of monocytic cells into the endothelium (57). Furthermore, PPAR-α agonists were shown to reduce the expression of endothelin-1. Endothelin-1 is a vaso-active peptide, involved in detrimental vasoconstriction and smooth muscle cell (SMC) proliferation (58). In SMCs, Staels *et al.* found that PPAR-α ligands inhibited interleukin IL-1-induced production of IL-6 and prostaglandin, and suppressed cyclooxygenase (COX)-2 activity by interfering with the nuclear factor-kappa bèta (NF-κβ) pathway (25). IL-1 and IL-6 are important cytokines involved in several cascades of inflammatory reactions. PPAR-α activators also inhibit monocytic tissue factor (TF) expression (59,60). TF is expressed on the surface of monocytes and macrophages in human atherosclerotic lesions and acts as the major procoagulant initiating thrombus formation in acute coronary syndromes (59). In the clinic, fibrate treatment reduces systemic levels of IL-6, the pro-inflammatory cytokine tumor necrosis factor (TNF-α), the prothrombotic factor fibrinogen, and the acute phase protein C-reactive protein (CRP). All of these inflammatory markers are associated with cardiovascular disease (25,61).

Taken together, by down-regulating several cytokines, peptides and proteins involved in vasoconstriction, SMC proliferation, monocyte activation, thrombosis, and inflammatory reactions, PPAR-α activators decrease vascular inflammation and consequently inhibit atherosclerosis. This is supported by the outcome of a number of clinical trials (see below).

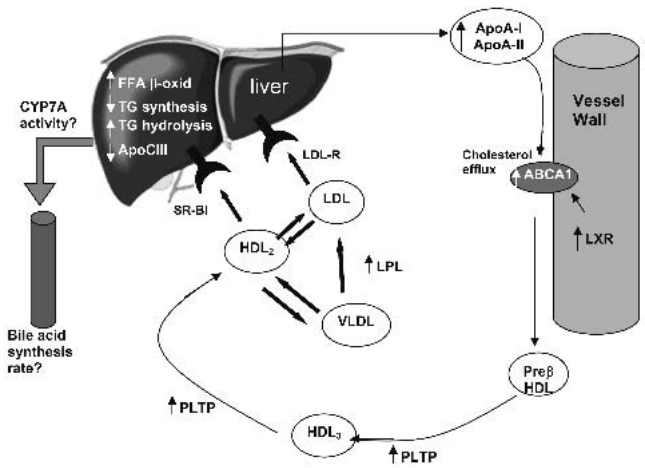

Fig. 4. The major actions of PPAR-α on lipid homeostasis and RCT.

CLINICAL USE OF PPAR-α AGONISTS

PPAR-α activators in the form of fibrates have been used to investigate the influence of their hypolipidemic effects on the incidence of CHD since 1966. In the first trials (62–66), clofibrate was the only drug available. After the fabrication of the second generation fibrates, new clinical trials were undertaken. In the last decade three angiographic endpoint trials were carried out: the Bezafibrate Coronary Atherosclerosis Intervention trial (BECAIT, 1996) (51), the Lopid Coronary Angiography Trial (LOCAT, 1997) (67) and the Diabetes Atherosclerosis Intervention Study (DAIS, 2001) (68). These studies showed that treatment with bezafibrate, gemfibrozil and fenofibrate, respectively, resulted in a significant reduction in lesion development and lumen narrowing in coronary arteries.

Three clinical end-point trials have been performed with second-generation fibrates. In the Helsinki Heart Study (1987), 2050 men with dyslipidemia were treated with gemfibrozil for five years. The trial demonstrated a 34% reduction in overall cardiac event rate (69). In *post hoc* analysis two important conclusions were drawn. First, it was shown that both LDL-C lowering and HDL-C elevating effects were independently correlated with a decrease in CHD incidence (70). Second, the patients who benefited most from gemfibrozil therapy (78% risk reduction) had a body mass index (BMI) > 26 kg/m^2, TG > 2.3 mmol/L or HDL-C < 1.08 mmol/L, and a fasting glucose > 4.4 mmol/L (71). Importantly, all these symptoms are grouped in the disease currently referred to as metabolic syndrome (MS), also known as Syndrome X. The more recent Veterans Affairs HDL Intervention Trial (VA-HIT) demonstrated a 22% risk reduction in the combined incidence of nonfatal myocardial infarction (MI) and CHD death after 5 years of gemfibrozil therapy (72). This cohort mainly included patients with low HDL-C levels as

most prominent lipid abnormality and contained a high number of patients with hyperinsulinemia and type II diabetes mellitus (DM). The Bezafibrate Prevention Study (BIP), however, showed a non-significant 9.4% reduction of nonfatal MI and CHD death in 3090 men and women treated with bezafibrate for 6 years (73). Only a small percentage of the patients included in this trial suffered from insulin resistance (IR) or type II DM.

However, a recent multicenter prospective study by Ohsawa *et al.* reported that 6 months of Bezafibrate therapy resulted in decreased total cholesterol and triglyceride and increased HDL cholesterol levels but without any improvement in the development of arterial disease (91).

Taken together, these clinical trials suggest that fibrate therapy protects against CHD, however, unlike the effects of multiple statins, the data are not very strong and total mortality benefit has yet not been demonstrated. However, the trials do show that fibrates are especially effective in patients with type II DM and MS. MS is defined as a clustering of several risk factors that include abdominal obesity, dyslipidemia (characterized by elevated TG and low HDL-C levels), raised blood pressure, insulin resistance, and a pro-inflammatory and prothrombotic state and often leads to type II DM and CVD (74). Approximately 24% of US adults have MS and the percentage is still rising, in parallel with the rising prevalence of obesity, making the disease an enormous health care problem (75). Currently two clinical endpoints studies in patients with diabetic dyslipidemia are in progress, in which the effectiveness of micronized fenofibrate (Fenofibrate Intervention and Event Lowering in Diabetes, FIELD) and atorvastatin (Collaborative Atorvastatin Diabetes Study, CARDS) are investigated. Hopefully, these trials will provide more insight into the specific indication of fibrate or statin therapy in this population.

PPAR-α POLYMORPHISMS

A common observation in fibrate-treated patients is the considerable variation in induced lipid changes, especially in LDL-C levels (76). There have also been a number of case reports of paradoxical decreases in HDL-C with second-generation fibrates (77,78). Genetic variation in the PPAR-α gene could be an important factor contributing to the different response to fibrate treatment. Several investigators have screened the PPAR-α locus for mutations and although a number of PPAR-α gene defects were described, most of them were either very uncommon or did not encode an amino acid change (79–83).

One polymorphism, however, was found with an allelic frequency of 6-8% in the general Caucasian population. It concerns a C→G transversion at position 484 in exon 5 which leads to a substitution of valine for leucine at codon 162 (L162V) in the DBD. Several researchers have investigated this polymorphism and have generated a vast amount of data.

Interestingly, two groups have demonstrated increased transcriptional activation of the PPAR-α L162V polymorphism in transfection studies. Both studies showed similar basal activation of the L162V variant compared to wild type (WT) PPAR-α, however, when a potent PPAR-α activator was added, the polymorphism induced up to 5 times higher expression (80,81).

Clinical data on the L162V polymorphism, however, are not conclusive. A number of laboratories have investigated the influence of this polymorphism on the response to fibrate treatment. Three studies reported no different response to gemfibrozil, fenofibrate, and bezafibrate treatment among carriers of the V allele and homozygotes for L162 (83–85). By contrast, Flavell *et al.* (81) found that among type II diabetics on bezafibrate, heterozygotes for V162 showed a 2-fold greater reduction of total cholesterol (TC) and non-HDL-C than homozygotes for L162. This was supported by data from another group, who reported a stronger decrease of non-HDL-C levels in response to fenofibrate treatment (86). However, this was only in combination with an apo-E2 genotype and did not reach statistical significance ($p = 0.09$). In another study, L162V carriers showed a 50% higher increase of HDL_2-C after gemfibrozil treatment compared to L162 homozygotes (87).

The data from association studies are not conclusive either. In healthy subjects, carriers of the V allele were associated with higher LDL-C, apo B-100, apo C-III, and TG plasma levels in two studies (82,88). By contrast, other association studies reported no significant difference in plasma lipid levels in healthy subjects (81,85,89). Flavell *et al.* found that, despite comparable lipid levels, carriers of the V allele had less atherosclerosis than L162 homozygotes, as measured by coronary angiography (85).

In addition, several groups have investigated this polymorphism in subjects with type II DM with again inconclusive outcome. The L162V polymorphism was associated with a lower BMI in one study (89) and with a more beneficial lipid profile with decreased TG, elevated HDL-C and apo A-I levels in two other studies (81,83). Two groups, however, found a pro-atherogenic lipid profile with elevated apo B-100 levels compared to non-carriers (79,82). Most recently, Brousseau *et al.* showed that healthy subjects carrying the V allele were at increased risk of developing a CHD event, whereas V-allele-carriers with type II DM or IR were at decreased risk. The risk reduction seen in L162V carriers with type II DM or IR could not be explained by beneficial changes in their lipid profile (90).

It remains to be determined whether the PPAR-α L162V polymorphism influences the response to fibrate therapy and the risk of CHD. This polymorphism seems to have more favorable effects in patients with type II DM than in healthy subjects, although the mechanisms underlying this have not been clarified. Further studies will be required to elucidate the precise impact of the L162V polymorphism on lipid profiles, the response to fibrates, and ultimately the risk of developing cardiovascular events in different populations.

CONCLUSIONS AND FUTURE PERSPECTIVES

Fibrates have been in use for a long time because of their hypolipidemic effects, but after the introduction of the more powerful statins, their use has been limited. Recently the interest in fibrates has been renewed. The first reason for this is their ability to elevate low HDL-C levels, which are a major risk factor for the development of CHD. Second, fibrates have shown to be particularly effective in dyslipidemic patients with type II DM or MS, diseases of which the prevalence in Western countries is rising swiftly and which often prelude CVD.

A novel generation of PPAR-α agonists is currently un-

der development, supported by the increased knowledge of PPAR-α structure and the mechanisms of action of fibrates. Because the present fibrates are relatively nonspecific ligands of PPAR-α, more specific designed PPAR-α activators could have increased efficacy and decreased adverse effects, especially in combination with statins. Also, co-activators of both PPAR-α and PPAR-γ could have a great potential. PPAR-γ is expressed in adipose tissue where it controls adipocyte differentiation. PPAR-γ agonists, the thiazolidinediones (TZDs), improve insulin sensitivity, lower glucose levels, and lower plasma TG and FFA levels by enhancing their uptake into adipocytes. Dual activation of both PPAR-α and PPAR-γ could have beneficial effects on a number of aspects of the metabolic disorders seen in type II DM and MS. These new compounds will have to demonstrate in clinical trials whether they are useful in the treatment of atherosclerosis and CVD.

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