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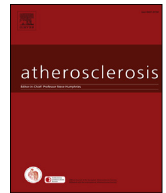

The novel selective PPARα modulator (SPPARMα) pemafibrate improves dyslipidemia, enhances reverse cholesterol transport and decreases inflammation and atherosclerosis

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Background: Atherosclerosis is characterized by lipid accumulation and chronic inflammation in the arterial wall. Elevated levels of apolipoprotein (apo) B-containing lipoproteins are a risk factor for cardiovascular disease (CVD). By contrast, plasma levels of functional high-density lipoprotein (HDL) and apoA-I are protective against CVD by enhancing reverse cholesterol transport (RCT). Activation of peroxisome proliferator-activated receptor-α (PPARα), a ligand-activated transcription factor, controls lipid metabolism, cellular cholesterol trafficking in macrophages and influences inflammation.
Objective: To study whether pharmacological activation of PPARα with a novel highly potent and selective PPARα modulator, pemafibrate, improves lipid metabolism, macrophage cholesterol efflux, inflammation and consequently atherosclerosis development in vitro and in vivo using human apolipoprotein E2 Knock-In (apoE2KI) and human apoA-I transgenic (hapoA-I tg) mice.
Approach and results: Pemafibrate treatment decreases apoB secretion in chylomicrons by polarized Caco-2/TC7 intestinal epithelium cells and reduces triglyceride levels in apoE2KI mice. Pemafibrate treatment of hapoA-I tg mice increases plasma HDL cholesterol, apoA-I and stimulates RCT to feces. In primary human macrophages, pemafibrate promotes macrophage cholesterol efflux to HDL and exerts anti-inflammatory activities. Pemafibrate also reduces markers of inflammation and macrophages in the aortic crosses as well as aortic atherosclerotic lesion burden in western diet-fed apoE2KI mice.
Conclusions: These results demonstrate that the novel selective PPARα modulator pemafibrate exerts beneficial effects on lipid metabolism, RCT and inflammation resulting in anti-atherogenic properties.
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1. Introduction

Atherosclerosis is a pathological process in which lipid deposition in the intima and media of the arterial wall promotes formation of plaques. Atherosclerotic plaques result from the progressive accumulation of cholesterol and diverse lipids in native and oxidized forms, extracellular matrix and inflammatory cells [1,2]. Atherogenic dyslipidemia, a cardiovascular risk factor for atherosclerosis, is an imbalance between pro-atherogenic apoB-containing lipoproteins (Chylomicrons and VLDL remnants, LDL) and anti-atherogenic apoA-I containing HDL. Reduction of atherogenic

Abbrevations: SPPARMα, selective PPARα modulator; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; PPAR, peroxisome proliferator-activated receptor; LPL, lipoprotein lipase; IL, interleukin; RCT, reverse cholesterol transport; MCP1, monocyte chemoattractant protein 1; TNFα, tumor necrosis factor alpha; TG, triglycerides; ABCA1, ATP-bindind cassette transporter A1; KI, knock-in; CMC, carboxy methyl cellulose; tg, transgenic; C, cholesterol; N-HDL-C, non-HDL cholesterol; TC, total cholesterol.

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dyslipidemia is therefore considered as a pharmacological approach to treat CVD [3].

Fibrates are used in the clinical management of dyslipidemia [4]. They lower triglycerides (TG) and, to differing extents, LDL-C and modestly increase HDL-C [5]. Fibrates activate a transcription factor belonging to the nuclear receptor superfamily, PPARα. PPARα is also activated by various natural ligands, like eicosanoids and fatty acids. Following ligand activation, PPARα heterodimerizes with the retinoid X receptor and binds PPAR response elements (PPRE) localized in the regulatory regions of target genes, thus regulating genes involved in many biological processes, such as lipid and glucose homeostasis [6,7]. Disruption of the PPARα gene in mice revealed its role in fatty acid oxidation, fatty acid uptake and lipoprotein assembly and transport [8]. Fibrates improve lipid and lipoprotein metabolism by reducing chylomicron secretion by enterocytes and increasing TG catabolism through up-regulation of lipoprotein lipase (LPL) [9] and its activator apoAV [10] and by down-regulation of apoC-III [11–13] an inhibitor of LPL activity. Moreover, fibrates increase intestinal and hepatic HDL production [14]. Expression of the major HDL apolipoproteins, apoA-I and apoA-II [15,16], is induced by fibrates. PPARα activators control also cholesterol homeostasis in macrophages [17]. Macrophages are able to eliminate excess of cholesterol by specific efflux pathways by inducing cholesterol transporters like ABC transporter 1 (ABCA1) [18] or scavenger receptor BI (SR-B1) [19] towards apoA-I and HDL. By this way, cholesterol is carried by HDL particles to the liver where it is metabolised, a process called reverse cholesterol transport (RCT). Mice overexpressing apoA-I display increased macrophage RCT [20], whereas mice deficient in apoA-I show reduced macrophage RCT [21]. In addition, PPARα also inhibits pro-inflammatory pathways by interfering with signaling pathways, such as nuclear factor (NF)-κ B and activator protein (AP)-1 [22] in a DNA-binding independent manner [23]. The impact of PPARα on inflammation and atherosclerosis has been largely studied. In vitro and ex vivo studies suggest that PPARα activation may inhibit various inflammatory pathways by decreasing the production of pro-inflammatory cytokines induced by NF-κB, like the interleukins IL1 and IL6 [24], as well as by repressing cytokine-induced expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) [25]. Studies using PPARα–deficient mice crossed with models of atherosclerosis [26–28] and bone marrow-derived macrophage transplant experiments demonstrated the anti-atherogenic effects of PPARα [29].

Clinical studies, such as the Lopid Coronary Angiographic Trials (LOCAT), Bezafibrate Atherosclerosis Coronary Intervention Trial (BECAIT) and Diabetes Atherosclerosis Intervention Study (DAIS), have shown that fibrate therapy (gemfibrozil, bezafibrate or fenofibrate) reduces angiographically-assessed atherosclerosis progression. Moreover, outcome trials, such as the Veterans Affairs-high density lipoprotein cholesterol Intervention Trial (VA-HIT), Helsinki Heart Study (HHS), Bezafibrate Infarction Prevention study (BIP), Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and Action to Control Cardiovascular Risk in Diabetes (ACCORD) indicate that the beneficial effects of fibrates are most pronounced in patients with elevated triglycerides and low HDL-C [4,30]. Nevertheless, some limitations of fibrate therapy are related to their weak activity on PPARα and their efficacy which depends on the targeted population. Novel compounds have been developed in the past decade, which are selective PPAR [31] modulators (SPPARMs) and have more potent PPARα agonist activity [32]. In this concept, the binding of PPAR ligands induces different conformational changes causing distinct patterns of cofactor recruitment and promoting selected biological responses [33].

Given the potential of PPARα activation to control residual cardio vascular risk, we tested the effect of a new potent selective PPARα modulator pemafibrate (EC_{50} on Gal4hPPARα = 1 nM), which is currently in phase 3 clinical development [34], on lipid and lipoprotein metabolism in apoE2KI mice, a model of atherogenic dyslipidemia, and in hapoA-I tg mice. In addition, the influence of pemafibrate on cholesterol efflux from macrophages towards HDL particles was studied in human primary macrophages. The effect of pemafibrate was further explored on RCT in hapoA-I tg mice. Finally, we examined the activity of pemafibrate on inflammation in macrophages, the vascular wall and on atherosclerosis in apoE2KI mice fed a western diet.

2. Materials and methods

2.1. GST pull down experiments

[^{35}S]-labelled PPARα was synthetized by using a Quick T7 TNT Kit (Promega). A total of 5–10 pmol of [^{35}S]-PPARα was incubated with vehicle (DMSO), or ligands (10 μmol/L) in binding buffer [20 mmol/L Tris-HCL (pH 7,5), 150 mmol/L NaCl, 10% glycerol and 0.1% Triton X-100]. After a 60 min incubation at 20 °C, cofactors expressed as a GST-fusion protein and adsorbed to a Sepharose-glutathione resin were added and agitated slowly on a rotating wheel for 90 min at 20 °C. Unbound material was removed by three successive washes of the Sepharose beads in 1× PBS-0.1% Triton X-100. Resin-bound receptors were then resolved by 8% SDS-PAGE and detected by autoradiography on PhosphorImager (Molecular Dynamics).

2.2. Cells and in vitro experiments

Caco-2/TC7 cells (from Pr Chambaz, Inserm 872, Paris, France) were routinely grown in complete medium (Dulbecco's modified essenial medium containing 25 mM glucose and glutamax; Gibco®). The medium was supplemented with 20% (v/v) of fetal calf serum, 1% (v/v) non-essential amino-acids and 1% (v/v) penicillin/streptomycin. For experiments, cells were cultured for 4 weeks on microporous membrane inserts in assymmetric conditions before activation with the PPARα ligands (GW7647 at 600 nM, Fenofibric acid at 50 μM or pemafibrate at 0.1, 1 and 10 μM) added in the apical compartment in serum free complete medium. Basolateral media was collected after 24 h, followed by apoB quantification by the ELISA method (Mabtech®, Nacha Strand, Sweden).

THP1 and J774 cells were purchased from American Type Culture Collection ATCC and cultured in RPMI1640 medium containing gentamycin (40 mg/ml), 1% (v/v) glutamine and 10% (v/v) fetal calf serum. THP1 cells were differentiated into macrophages by exposure to phorbol-12myristate-13-acetate (PMA, Promega). THP1 macrophages were stimulated with LPS (100 ng/mL) in presence or not of PPARα ligands (Fenofibric acid or pemafibrate), and secreted cytokine levels were measured 24 h later with ELISA kits according to the manufacturer's instructions (R&D System). Cytokine mRNA levels were determined by quantitative PCR analysis.

J774 cells were grown in suspension in RPMI 1640 supplemented with 10% fetal calf serum and gentamycin (40 mg/mL). LDL was radiolabeled with 5 μCi/ml [^{3}H]-cholesterol and cells were loaded with 100 μg/mL of acetylated LDL for 48 h.

Mononuclear cells isolated from blood of healthy normolipidemic donors by Ficoll gradient centrifugation and suspended in RPMI 1640 medium containing gentamycin (40 mg/mL), glutamine 0.05% (v/v) and 10% (v/v) pooled human serum. Differentiation of monocytes into macrophages occurs spontaneously by adhesion of the cells to the culture dishes. Mature monocytes-derived macrophages were used for experiments after 10 days of culture. Culture medium was changed to serum-deprived medium prior to treatment. Human primary macrophages were treated for 3 days with

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