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

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Article history:
Received 7 December 2015
Received in revised form 19 February 2016
Accepted 3 March 2016
Available online 4 March 2016

Keywords:
SPPARMα
Lipids
Inflammation
Reverse cholesterol transport
Atherosclerosis
Pemaflibrate

A B S T R A C T

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, pemaflibrate, 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: Pemaflibrate treatment decreases apoB secretion in chylomicrons by polarized Caco-2/TC7 intestinal epithelium cells and reduces triglyceride levels in apoE2KI mice. Pemaflibrate treatment of hapoA-I tg mice increases plasma HDL cholesterol, apoA-I and stimulates RCT to feces. In primary human macrophages, pemaflibrate promotes macrophage cholesterol efflux to HDL and exerts anti-inflammatory activities. Pemaflibrate 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 pemaflibrate 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
dyslipidemia is therefore considered as a pharmacological approach to treat CVD [3]. Fibrate 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]. Fibrate activate a transcription factor belonging to the nuclear receptor superfamily, PPARβ. PPARs are also activated by various natural ligands, like eicosanoids and fatty acids. Following ligand activation, PPARs heterodimerize 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 PPARβ gene in mice revealed its role in fatty acid oxidation, fatty acid uptake and lipoprotein assembly and transport [8]. Fibrate 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 fibrate. PPARβ 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, PPARβ 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 PPARβ on inflammation and atherosclerosis has been largely studied. In vitro and ex vivo studies suggest that PPARβ 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 PPARβ-deficient mice crossed with models of atherosclerosis [26–28] and bone marrow-derived macrophage transplant experiments demonstrated the anti-atherogenic effects of PPARβ [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 fibrate 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 PPARβ activation to control residual cardio vascular risk, we tested the effect of a new potent selective PPARβ modulator pemafibrate (EC50 on Gal4bPPARβx = 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 hapa0-A1 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 hapa0-A1 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

[35S]-labelled PPARβ was synthesized by using a Quick T7 TNT Kit (Promega). A total of 5–10 pmol of [35S]-PPARβ 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 essential 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) glucose and 10% (v/v) fetal calf serum. THP1 cells were differentiated into macrophages by exposure to phorbol-12myristate-13-acetae (PMA, Promega). THP1 macrophages were stimulated with LPS (100 ng/ml) in presence or not of PPARα ligands (Fenofibrin 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 [3H]-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), glucose 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
the PPARα ligands (Fenofibric acid at 100 μM or pemafibrate at 0.1, 1, 5 and 10 μM) and mRNA levels of ABCA1, ABCG1 were determined by quantitative PCR analysis. In additional experiments, primary human macrophages were loaded with [3H]-cholesterol-containing AcLDL (50 μg/mL, containing [3H]-cholesterol) for 48 h. Fenofibric acid at 100 μM or pemafibrate at 1, 5, 10 μM were added 24 h before and during cholesterol loading. Cells were then incubated in the presence or absence of HDL particles and [3H] cholesterol efflux was measured.

2.3. Animal experiments

Female homozygous human apoE2KI mice, which express human apoE2 instead of mouse apoE [35] and display a human-like lipoprotein profile, were fed a western diet containing (wt/wt) 0.2% cholesterol and 21% fat (UAR, Epinay sur Orge, France) for 9 weeks and treated for the last 2 weeks with fenofibrate (250mpk) or pemafibrate (0.1 or 1mpk) or carboxy methyl cellulose (CMC, control) (n = 10/group). Blood was collected after 4 h-fasting by sinus retroorbital puncture under isoflurane-induced anaesthesia for biochemical analysis. Mice were euthanized by cervical dislocation and liver, aortic arch and intestinal epithelium were removed and snap-frozen until further analysis.

For analysis of atherosclerotic lesions, male mice (n = 9–10/group) were fed a western diet supplemented or not (control group) with fenofibrate or pemafibrate for 10 weeks. Hearts were fixed with 4% phosphate-buffered paraformaldehyde (pH 7.0) and serial 10-μm-thick sections were cut between the valves and the aortic arch for quantitative analysis of lipid deposition by oil red-O staining. Images were captured with a JVC 3-charge-coupled device video camera. Sections were analyzed using the computer-assisted Quips Image analysis system (Leica Mikroskopic und System GmbH, Wetzlar, Germany).

Female human apoA-I tg mice (n = 6/group) were treated with fenofibrate at 250mpk or pemafibrate at 0.1 or 1mpk for 2 weeks. Blood was collected after 4 h-fasting for biochemical analysis. In another experiment, mice were treated with fenofibrate at 250mpk or pemafibrate at 1mpk or CMC (control) during 2 weeks before intraperitoneal injection of radiolabeled [3H]-cholesterol radiolabeled macrophages. Feces were collected during 48 h and were stored at −20 °C before fecal cholesterol extraction.

2.4. Lipid and lipoprotein analysis

Plasma levels of total cholesterol (TC) and triglycerides (TG) were measured using commercially available kits (Boehringer-Mannheim, Germany; BioMérieux, France respectively). High density lipoprotein-cholesterol (HDL-C) concentrations were determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid/Mg (Boehringer-Mannheim, Germany). Non-HDL cholesterol (N-HDL-C) values were calculated by subtracting HDL-cholesterol from total cholesterol.

Lipoprotein cholesterol distribution profiles were obtained by gel filtration chromatography using a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech) on pooled plasma samples from each group.

2.5. Fecal cholesterol extraction

Feces collected from 0 to 48 h were weighed and fecal cholesterol were extracted. Results are expressed as counts per min in total feces by wet weight.

2.6. RNA extraction and analysis

Total RNA was extracted from livers and aortic crosses by using the acid guanidinium isothiocyanate/phenol/chloroform method or from cells or scrapped intestinal epithelium using Extract All reagent. For quantitative PCR, RNA was reverse transcribed using random hexamer primers and Moloney murine leukemia virus-reverse transcriptase (Applied Biosystems®). RNA levels were determined by real time quantitative polymerase chain reaction on MX-3000 apparatus (Agilent®) and specific primers (Eurogentec®) (Suppl. Table I). Gene expression levels were expressed as mean ± SD relative to cyclophilin.

2.7. Statistical analysis

Statistical significance was determined by using a Student’s t-test for real-time quantitative PCR and ELISA assays and by using and ANOVA followed by the post hoc Sheffe test for animal experiments. P values < 0.05 were considered as significant and different levels of significance are represented by asterisks: *p < 0.05, **p < 0.01, ***p < 0.001.

3. Results

3.1. Pemafibrate improves lipoprotein metabolism

To investigate the effects of pemafibrate on plasma lipids, homozygous human apoE2KI displaying mixed dyslipidemia, were fed a western diet during 7 weeks and subsequently treated with or without fenofibrate (250mpk, the highest tolerable preclinical dose) or pemafibrate for 2 weeks. Compared to control mice, pemafibrate at 0.1 and 1mpk strongly reduced plasma TC (−72% and −79% respectively, p < 0.001) and TG (−68% and −85%, p < 0.001) concentrations with a decrease of N-HDL-C levels (−76% and −87%, p < 0.001) (Fig. 1). The reference compound fenofibrate, given at a maximal efficacious dose of 250mpk, also lowered plasma lipids but to a lesser extend (TC, −58%, p < 0.001; TG, −18%, p < 0.05 and N-HDL-C, −61%, p < 0.001). ApoB mRNA levels, coding for an apolipoprotein essential for the assembly of chylomicrons and very low density lipoproteins (VLDL) in the intestine and the liver respectively, decreased gradually upon treatment with pemafibrate at 1mpk along the intestinal track [duodenum −75% (p < 0.001), jejunum −60% (p < 0.001), ileum −41% (p < 0.01)], while fenofibrate reduced apoB mRNA by only 42% in the duodenum (p < 0.01) (Fig. 2). Hepatic apoB mRNA levels did not change upon treatment with either fenofibrate or K877 (data not shown).

To test for a direct effect on apoB secretion by intestinal epithelial cells, polarized Caco-2/TC7 cells grown and polarized on a porous filter to mimic the human intestinal barrier, were treated with the different PPARα ligands added to the apical side. After 24 h, medium in the basolateral compartment was collected, chylomicrons isolated by centrifugation and apoB quantified by ELISA. Fenofibric acid (50 μM) slightly reduced (−18%) apoB secretion in the medium. GW7647, another synthetic PPARα agonist (600 nM) and pemafibrate (10 μM) significantly decreased apoB secretion [−30% (p < 0.05) and −73% (p < 0.01), respectively] (Fig. 2C).

Moreover, hepatic mRNA levels of apoC-III, an inhibitor of LPL activity controlling TG clearance, decreased by 38% (p < 0.001) and 61% (p < 0.001) with pemafibrate at 0.1mpk and 1mpk respectively. Fenofibrate decreased apoC-III mRNA by 36% (p < 0.01) (Fig. 2B). HDL-C levels strongly increased upon pemafibrate at 0.1mpk and 1mpk (+104%, p < 0.01 and + 252%, p < 0.001), while fenofibrate only induced a 75% (p < 0.01) increase of HDL-C in apoE2KI mice (Fig. 1). In human apoA-I tg mice, a mouse model in which plasma cholesterol is mainly carried by HDL particles, pemafibrate and
**Fig. 1.** Influence of pemafibrate and fenofibrate on plasma lipid concentrations in apoE2KI mice fed a western diet. ApoE2KI mice were fed a western diet during 7 weeks followed by 2 additional weeks on western diet, treated or not with fenofibrate or pemafibrate at the indicated doses. Plasma lipid concentrations were measured at the end of the treatment period. Values are expressed as mean±SD. *p < 0.05, **p < 0.01, ***p < 0.001.

**Fig. 2.** Effects of pemafibrate, GW647 and fenofibrac acid on mRNA levels of genes involved in triglyceride metabolism in the liver and intestine of apoE2KI mice and on apoB secretion by polarized Caco-2/TC7 cells. mRNA levels of intestinal apoB (A) and hepatic apoC-III (B) were determined by quantitative PCR analysis. White bar, control (C) group; black bar, fenofibrate (F) (250mpk) treated mice; hatched bar, pemafibrate at 0.1mpk; grey bar, pemafibrate at 1mpk. Data are presented relative to control. Chylomicrons were isolated by ultracentrifugation followed by apoB quantitation (ELISA) in polarized Caco-2/TC7 treated cells (C). White bar, control (C) cells; hatched bar, GW7647 (GW) (600 nM); black bar, fenofibrac acid (FA) (50 μM) treated cells; hatched bar, pemafibrate at the indicated doses. *p < 0.05, **p < 0.01, ***p < 0.001.
Pemafibrate significantly induced TC (+409%, p < 0.001; 248%, p < 0.001, respectively) (Fig. 3A). Separation of the different lipoproteins by gel filtration confirmed the robust increase of cholesterol in HDL particles, with a stronger effect observed with pemafibrate at 1mpk compared to fenofibrate at 250mpk (Fig. 3B). Increased HDL-C was associated with a concomitant increase in plasma human apoA-I upon pemafibrate and fenofibrate treatment (+194%, p < 0.001; +146%, p < 0.001, respectively) (Fig. 3A).

### 3.2. Pemafibrate promotes cholesterol efflux in vitro and reverse cholesterol transport (RCT) in human apoA-I tg mice

To determine the activity of pemafibrate on cholesterol trafficking, human primary macrophages were treated with the PPARα ligands and the expression of the cholesterol transporters adenosine triphosphate (ATP)-binding cassette transporters ABCA1 and ABCG1 quantified. Cholesterol efflux to HDL particles was also assessed in labelled cholesterol-loaded macrophages. Pemafibrate strongly induced ABCA1 (+563%, p < 0.01 at 10 μM) and ABCG1 (+2093% p < 0.001 at 10 μM) mRNA steady-state levels in a dose-dependent manner, being again more active than fenofibrate (+168%, p < 0.05 for ABCA1 and +506%, p < 0.01 for ABCG1) (Fig. 4A). Moreover, pemafibrate more pronouncedly induced cholesterol efflux to HDL compared to fenofibrate (1.9-fold, p < 0.05 vs 1.6-fold, p < 0.05 respectively) (Fig. 4B). Finally, pemafibrate significantly increased fecal [3H]-lipid excretion (p < 0.05), while fenofibrate only had a marginal effect (NS) in hapoA-I tg mice intraperitoneally injected with labelled macrophage foam cells.

### 3.3. Pemafibrate has anti-inflammatory activities in macrophages in vitro and in the aorta in vivo

To determine the anti-inflammatory activity of pemafibrate, mRNA and protein expression of diverse chemo- and cytokines, like monocyte chemoattractant protein 1 (MCP1), interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα), were measured in human THP-1 macrophages treated with the different PPARα ligands prior to LPS-activation. Pemafibrate dose-dependently repressed MCP1 (−90%, p < 0.001) and IL6 (−95%, p < 0.01) mRNA levels, while fenofibrate (at 100 μM) showed intermediate efficiency (−56%, p < 0.001 for MCP1 and −60%, p < 0.01 for IL6) (Suppl. Fig. 1A). Similarly, secretion of MCP1 and IL6 also decreased upon incubation with pemafibrate and fenofibrate (around −90%, p < 0.001 for pemafibrate as compared to −50%, p < 0.05 for fenofibrate) (Suppl. Fig. 1B). In addition, TNFα secretion, but not mRNA, was reduced by pemafibrate (−85%, p < 0.001) (Suppl. Fig. 1). To determine the anti-inflammatory activity in vivo, vascular inflammation was determined in apoE2KI mice fed a western diet supplemented or not with pemafibrate or fenofibrate during 10 weeks. Expression of VCAM-1, the macrophage marker F4/80, MCP1 and IL6 decreased in pemafibrate treated mice (at 1mpk, −32%, p < 0.01 for VCAM-1, −30%, p < 0.01 for F4/80, −21%, ns for MCP1 and −40%, p < 0.05 for IL6), while fenofibrate had only a minor effect (NS) on these parameters (Fig. 5A). As in previous experiments, a higher efficacy of pemafibrate was observed as compared to fenofibrate.
3.4. Pemafibrate decreases atherosclerotic lesions in western diet-fed apoE2KI mice

To assess whether pemafibrate treatment influences atherosclerosis, atherosclerotic lesion areas were measured by Oil-Red-O lipid staining in apoE2KI mice fed a western diet and treated during 10 weeks with pemafibrate at 0.1mpk and 1mpk. Both doses of pemafibrate significantly reduced atherosclerotic lesion areas by 71% and 89% respectively as compared to control mice (median: 0.061 mm² at 0.1mpk and 0.022 mm² at 1mpk versus 0.207 mm² in control mice; p < 0.001) indicating a dose-response effect of pemafibrate treatment (Fig. 5B and C). Fenofibrate reduced atherosclerotic lesion sizes to a slightly lower extend than pemafibrate (median: 0.06 mm² in fenofibrate treated mice vs 0.207 mm² in control mice; p < 0.001) (Fig. 5B and C).

3.5. Pemafibrate induces a distinct co-factor recruitment profile compared to fenofibrate

To determine whether the different activities of pemafibrate and fenofibrate may be related to ligand-specific conformational changes of PPARα resulting in formation of coactivator complexes, GST pull-down experiments were carried out (Suppl. Fig. II). Pemafibrate more efficiently promoted the recruitment of co-activators SRC-1, PGC1α and GRIP1 than fenofibric acid, whereas DRIP205 was recruited to a similar extent.

4. Discussion

PPARα agonists such as fibrates are useful drugs in the treatment of dyslipidemia characterized by elevated triglycerides and reduced HDL-C levels [30]. The effect of PPARα agonists on TG lowering is mainly attributed to the regulation of genes involved in lipid absorption, trafficking and metabolism. The intestine and the liver are major organs participating in the synthesis of apolipoproteins and the production of lipoproteins. ApoB is present exclusively in atherogenic lipoproteins such as chylomicrons, VLDL, IDL and LDL which carry cholesterol to peripheral tissues. PPARα activation not only decreases the secretion of apoB by enterocytes [14], but also promotes TG catabolism by enhancing fatty acid oxidation and inducing LPL gene transcription in liver and muscle [9]. In addition, PPARα agonists down-regulate the expression of the LPL inhibitor apoC-III [11,13], and induce the LPL activator apoA-V [10]. ApoA-I is the major apolipoprotein of HDL particles, which promote cholesterol efflux from peripheral tissues to the liver, from where it is excreted into the bile and eliminated into feces, a process called RCT. PPARα agonists induce hepatic apoA-I synthesis and HDL production [8,15,16]. Moreover PPARα activation controls cholesterol trafficking in human macrophages [17] and induces the expression of cholesterol transporters ABCA1, ABCG1 in macrophages. As a result, PPARα activation enhances the first step of RCT. Altogether these activities are thought to be atheroprotective. In this study, we characterized the pharmacodynamic activity of a novel potent and SPPARMα pemafibrate, currently in phase 3 clinical trials [34], on lipid metabolism by using humanized mouse models of mixed dyslipidemia (apoE2KI mice) and elevated HDL.
(human apoA-I tg mice) and compared its activity to fenofibrate, the clinically used reference compound, tested at its highest pre-clinical dose. In apoE2KI mice, pemafibrate markedly decreases TG and N-HDL-C. Moreover, pemafibrate decreases apoB expression in the intestine through a direct action on enterocytes as shown using polarized Caco2/TC7 cells. Moreover, pemafibrate decreases apoC-III gene expression in the liver, which suggests an increased lipolytic activity which can contribute to the TG-lowering effect of this compound. In Sprague-Dawley rats, it has been demonstrated that daily treatment with pemafibrate at a dose of 1mpk or fenofibrate at 100mpk equivalently reduce TG concentrations by about 50% [35]. This TG-lowering effect is associated with a decrease of two LPL-regulating factors, apoC-III and angiopoietin-like 3 (ANGPTL3) resulting in a 2-fold increased plasma LPL activity [35]. Beside accelerating TG clearance, pemafibrate also reduced in rats de novo TG synthesis by 72% as well as the expression of the lipogenic genes FAS, ACC and DGAT2. Furthermore, fatty-acid β-oxidation increased upon pemafibrate treatment [35]. Altogether these observations provide an explanation for the TG-lowering activity of pemafibrate.

HDL-C concentrations increased in apoE2KI and human apoA-I tg mice upon pemafibrate treatment. The increase of HDL-C was associated with an increase of human apoA-I concentrations. Our data on TG and HDL-C are in line with results from phase II/III clinical studies showing that pemafibrate significantly improves plasma lipoprotein profiles in Japanese patients with dyslipidemia [36]. Among the HDL particles, small HDL (HDL3) have been reported to be more efficient than large HDL (HDL2) in RCT [37,38] and the shift in lipoprotein subclass distribution could contribute to reduce the risk of atherosclerosis and coronary heart disease in man. Pemafibrate treatment (12 weeks) of Japanese patients caused a shift in HDL composition towards smaller particles increasing HDL3-C levels [39]. ApoA-I and HDL promote excess cholesterol efflux from peripheral tissues and transport towards the liver after which cholesterol is excreted into the bile and feces [20]. Clinical [38,40] and transgenic mouse model [21] studies have established that HDL function is inversely correlated with cardiovascular disease. Moreover, recent studies showed that efflux capacity of HDL, rather than its cholesterol content, determines its atheroprotective activity [41]. In human primary macrophages, pemafibrate and fenofibrate promote cholesterol efflux towards HDL particles by increasing expression of the ABCA1 and ABCG1 cholesterol transporters. To examine the functionality of apoA-I and HDL induction by pemafibrate and fenofibrate on RCT, [3H]-cholesterol labelled macrophage foam cells were intraperitoneally injected in hapoA-I tg mice. Macrophage-to-feces RCT assessment showed that pemafibrate enhances the fecal excretion of
cholesterol, likely through induction of apoA-I and HDL particles as shown by Rader et al. [20], just like in hapoA-I tg mice treated with fenofibrate [42].

Numerous studies in PPARα-deficient mice have implicated PPARα in the control of the inflammatory response. In PPARαKO mice, levels of inflammatory markers, such as VCAM1, are increased in murine endothelial cells [43], aortas display an exacerbated inflammatory response to LPS stimulation [22]. Furthermore, the response to inflammatory stimuli in macrophages is enhanced [29]. Fenofibrate treatment repressed LPS induction of inflammatory cytokines like MCP1, IL6 and TNFα fed a western diet to promote atherosclerosis development, inflammatory and macrophage makers are also decreased by pemafibrate in the aortas. In these mice, pemafibrate and fenofibrate also reduce the atherosclerotic lipid burden. It is important to note that in all experiments lower doses of pemafibrate than of fenofibrate were used, and that, nevertheless, pemafibrate showed a higher efficacy than fenofibrate. Co-factor recruitment assays showed more efficient association of co-activators SRC-1, PGC1α and GRIP1, in the presence of pemafibrate compared to fenofibric acid, while recruitment of DRIP 205 with pemafibrate was similar to those with fenofibric acid indicating that it functions as a SPPARM which may contribute to the differential biological activities of pemafibrate measured in vitro and in vivo. Therefore, we conclude that the highly potent selective PPARα modulator pemafibrate exerts protective anti-atherogenic properties in mice by its TG and remnant lipoprotein-lowering effect, its beneficial effect on LDL metabolism and RCT, and its anti-inflammatory activity in macrophages and the arterial wall resulting in reduced atherosclerosis burden. Thus, pemafibrate is an interesting novel SPPARMz to treat atherogenic dyslipidemia and cardiovascular disease risk.

Sources of funding
This work was supported by grants from KOWA Company.

Disclosures
None.

Acknowledgments
We thank Dr Hafid Mezdour for providing the apoE2KI mouse strain.

Appendix A. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.03.003.

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