Peroxisome Proliferator-Activated Receptor α–Independent Actions of Fenofibrate Exacerbates Left Ventricular Dilation and Fibrosis in Chronic Pressure Overload

Toni-Ann S. Duhaney, Lei Cui, Mary K. Rude, Nathan K. Lebrasseur, Soeun Ngoy, Deepa S. De Silva, Deborah A. Siwik, Ronglih Liao, Flora Sam

Abstract—Progressive cardiac remodeling is characterized by subsequent chamber hypertrophy, enlargement, and pump dysfunction. It is also associated with increased cardiac fibrosis and matrix turnover. Interestingly, peroxisome proliferator-activated receptor (PPAR) α activators reduce cardiac hypertrophy, inflammation, and fibrosis. Little is known about the role of fenofibrates in mediating PPARα-independent effects in response to chronic pressure overload (PO). Wild-type and PPARα-deficient mice were subjected to chronic PO caused by ascending aortic constriction to test the role of fenofibrates in chronic, progressive cardiac remodeling by a PPARα-independent mechanism. Mice were randomized to regular chow or chow-containing fenofibrate (100 mg/kg of body weight per day) for 1 week before and 8 weeks after ascending aortic constriction. In the presence of PPARα, wild-type chronic PO mice, treated with fenofibrate, had improved cardiac remodeling. However, PO PPARα-deficient mice treated with fenofibrate had increased mortality, significantly adverse left ventricular end diastolic (3.4±0.1 versus 4.2±0.1 mm) and end systolic (1.5±0.2 versus 2.5±0.2 mm) dimensions, and fractional shortening (57±3% versus 40±3%). Fenofibrate also increased myocardial hypertrophy, cardiac fibrosis, and the ratio of matrix metalloproteinase-2/tissue inhibitor of matrix metalloproteinase-2 in PO PPARα-deficient mice. Fenofibrate inhibited matrix metalloproteinase activity in vitro and aldosterone-induced increases in extracellular signal-regulated kinase phosphorylation. Thus, fenofibrate improved cardiac remodeling in chronic PO mice. However, in PPARα-deficient mice, this chronic PO was exacerbated and associated with increased myocardial fibrosis and altered matrix remodeling. In the absence of PPARα, fenofibrates exerts deleterious, pleiotropic myocardial actions. This is an important observation, because PPARα agonists are considered possible inhibitory regulators of cardiac remodeling in the remodeled heart. (Hypertension. 2007; 49:1084-1094.)

Key Words: fibrosis ■ pressure overload ■ ventricular remodeling ■ peroxisome proliferator-activated receptor ■ matrix metalloproteinases

Myocardial remodeling caused by chronic hemodynamic overload is characterized by increased myocardial hypertrophy, which, although initially compensatory, is an unfavorable component of cardiac remodeling when it becomes progressive. Progressive left ventricular (LV) remodeling is characterized by subsequent chamber enlargement and pump dysfunction. These alterations occur together with increased expression of inflammatory cytokines,1 a shift from myocardial fatty acid oxidation to glucose use,2 constant myocardial extracellular matrix turnover,3 and matrix metalloproteinase (MMP) activation.4 Aldosterone plays a key role in mediating adverse LV remodeling with pressure overload (PO).5 It increases myocardial fibrosis that is independent of tissue hypertrophy and arterial pressure.6,7 Some of these effects are mediated directly by aldosterone, inducing MMP-2 and MMP-9 activities in cardiac myocytes. The mechanism partially involves activation of the MAP kinase/extracellular signal-regulated kinase (ERK)1/2 pathway.8

The benefits of targeted therapies to ameliorate progressive cardiac remodeling remain to be determined. In particular, the peroxisome proliferator-activated receptor (PPAR) family and their target genes appear to contribute in regulating lipid and glucose homeostasis and are implicated as regulators of inflammation and atherosclerosis.9 Three ligand-activated transcription factors have been identified: PPARγ, PPARβ/δ and PPARα. PPARγ is predominantly expressed in adipose tissue and regulates metabolism. PPARβ/δ is ubiquitously expressed10 and regulates fatty acid oxidation genes.9 In addition, PPARβ/δ activators exert anti-inflammatory and
antihypertrophic effects in cardiomyocytes.10 In contrast, PPARα is abundant in tissues with high rates of mitochondrial fatty acid oxidation, (ie, heart, liver, and kidney) and regulates genes involved in cellular lipid catabolism. PPARα, the main isoform in the adult heart,11 is downregulated in PO hypertrophy,12 and its dysregulation is associated with cardiac dysfunction.13 Conversely, PPARα ligands reduce cardiac hypertrophy and inflammation14,15 and, in acute PO, attenuate cardiac fibrosis.9 Finally, mice lacking PPARα are reportedly resistant to pleiotropic actions of PPARα ligands.16

Fibroblasts are predominantly PPARα but are also PPARβ/δ activators.17 Fenofibrates are used clinically for dyslipidemia and hypertriglyceridemia, and it is through the activation of PPARα that lipids are lowered. Likewise, fibroblasts have been considered as possible therapeutic agents for the treatment of cardiac hypertrophy18 and failure. Independent of their lipid-lowering actions, fenofibrates, as PPARα agonists, may act as negative regulators of proinflammatory genes by antagonizing the activity of inflammatory transcription factors (eg, nuclear factor κB19,20 and activator protein–121), thus decreasing myocardial inflammation.22 Likewise, inhibiting chronic inflammation in the remodeled myocardium is associated with a reduction in fibrosis.9,15,23

In the present study, we tested the hypothesis that fenofibrate exerts beneficial effects on chronic, progressive cardiac remodeling and hypertrophy by PPARα-independent actions. The present studies were aimed at determining the role of fenofibrate in the pathogenesis of cardiac remodeling using both in vivo (wild-type and PPARα-deficient mice) and in vitro studies (cultured adult rat cardiomyocytes).

Methods

Animals

There were 2 groups of mice. Group A wild-type (WT) FVB mice were purchased from Charles River Laboratories (Wilmington, Mass) and group B PPARα-deficient mice (129S4/SvJae- Ppara<sup>−/−</sup>/MjJ) were purchased from Jackson Laboratories (Bar Harbor, Maine). Study protocols were approved by the institutional animal care and use committee at Boston University.

Surgery

Both groups of mice underwent ascending aortic constriction (AAC) surgery as described previously.8 Briefly, 12- to 14-week–old mice were anesthetized with pentobarbital (15 mg/kg IP injection) and then ventilated on a Harvard rodent respirator (model 683). Aortic constriction was performed by AAC with a 7–0 silk suture around a 27-gauge needle via an anterolateral thoracotomy. This technique provided a minimal variability of the trans-constriction pressure gradients in mice.24 Sham-operated mice underwent a similar procedure without AAC.

Treatment

In group A, 32 mice (10 sham-operated and 22 banded) and, in group B, 50 mice (10 sham and 40 banded) were studied. Mice were randomly assigned to regular chow or chow-containing fenofibrate (100 mg/kg of body weight per day) for 1 week before and 8 weeks after surgery. A similar dose of fenofibrate was used by others25–27 and shown to be nontoxic. Echocardiogram, blood pressure (BP), histology, and cardiac protein analyses were performed 8 weeks after surgeries.

Echocardiography and BP Measurement

Transsthoracic echocardiography was performed in conscious mice before surgery (baseline) and 2, 4, and 8 weeks after surgery. Echocardiography was performed as described previously24 using an Acuson Sequoia C-256 echocardiograph machine and a 15-MHz probe. Tail cuff systolic BP and heart rate (HR) were measured at 8 weeks post-AAC, in group B, using a noninvasive tail cuff system (BP-2000, VisiTech) as described previously.5,28,29

Organ Weight

Mice were euthanized 8 weeks postoperatively. The body weight, LV heart weight, and wet and dry lung weights were determined. The wet/dry ratio was taken as an indicator of pulmonary congestion and clinical heart failure.30

Fibrosis

To measure fibrosis, trichrome-stained sections (5 μm) were visualized by light microscopy, and the entire section was quantified using Bioquant Image analysis software.

Western Blot Analysis

Hearts from PPARα-deficient mice were removed and snap frozen in liquid nitrogen. The tissue was homogenized in cold lysis buffer (10× cell lysis buffer, Cell Signaling) with the addition of 1 mmol/L of phenylmethylsulfonyl fluoride, 1 mmol/L of sodium orthovanadate, and 1 μg/mL of aprotinin. The Bradford method (Bio-Rad) was used to measure protein concentration. SDS-PAGE was performed under reducing conditions on both 10% and 12% separation gels with a 4% stacking gel (Cambrex). Proteins were transferred to Immun-Blot polyvinylidene fluoride membrane (Bio-Rad). Blots were incubated with primary antibodies to the PPARγ (Santa Cruz Biotechnology Inc), PPARβ/δ, MMP-9, tissue inhibitor of MMP (TIMP)-1, MMP-2, and TIMP-2 (Chemicon) for 18 to 20 hours at 4°C. Blots were then incubated in horseradish peroxidase–conjugated secondary antibody, and the signal was detected by SuperSignal West chemiluminescence (Pierce). All of the blots were normalized with Coomassie Brilliant Blue staining of the gels (Sigma Aldrich).

Isolation of Adult Rat Ventricular Myocytes

As described previously,8 adult rat ventricular myocytes (ARVMs; 90% to 95% purity) were isolated from the hearts of adult Sprague–Dawley rats, plated at a nonconfluent density of 30 to 50 cells/mm<sup>2</sup> on plastic culture dishes (Fisher) precoated with laminin (1 μg/cm<sup>2</sup>, Invitrogen), and maintained in ACCM medium (DMEM; BSA, 2 mg/mL; L-carnitine, 2 mmol/L; creatinine, 5 mmol/L; taurine, 5 mmol/L; penicillin, 100 IU/mL; and streptomycin, 10 g/mL) for 16 hours before drug treatment.

Drug Treatments

Myocytes were treated with aldosterone (50 nM, Sigma) for 30 minutes for signaling and 24 hours forzymography. Fenofibrate (10 μM and 100 μM, Sigma) was added 30 minutes before aldosterone.

ERK Phosphorylation

As described previously,8 ARVMs were collected in lysis buffer. Protein concentration was determined using Bradford assay (BioRad). Protein was probed with anti–phospho-ERK1/2 (1:1000, Cell Signaling) or anti–total-ERK1 (1:1000, Cell Signaling) antibodies. Chemiluminescence was quantified by densitometry (Molecular Analyst, Bio-Rad).

Assessment of MMP Activity

Using in-gel zymography as described previously,31 briefly ARVMs were treated for 24 hours in 100-nm dishes in medium without albumin. Conditioned medium was concentrated, and protein was determined by the Bradford assay.32,33 MMP activity was measured by in-gel zymography with gelatin (type A from porcine skin, Sigma) as the substrate. Unstained, digested regions representing
MMP activity were quantified using an imaging densitometer (GS700, Bio-Rad).

Statistical Analysis

All of the data are presented as mean±SEM. Differences between mice were tested for statistical significance by the 2-tailed Student t test or a 2-fold ANOVA with Bonferroni correction. Survival data were calculated using the Kaplan–Meier survival analysis. Similarly, in in vitro studies, differences among conditions were determined by ANOVA followed by a paired t test with the Bonferroni correction for multiple comparisons. P<0.05 was considered statistically significant.

Results

In Vivo Studies

Fenofibrate Effects on LV Structure and Function in WT Mice

Total wall thickness was increased by 2 weeks (1.22±0.09 mm versus sham: 0.99±0.02 mm; P<0.01), persisted at 4 weeks post-AAC, and slightly increased further by 8 weeks (Figure 1A). In addition, AAC caused progressive dilation with increases in LV end diastolic dimension and LV end systolic dimension that were evident at 2 weeks (3.37±0.09 and 1.97±0.01 mm, respectively), present at 4 weeks, and increased further at 8 weeks (Figure 1B and 1C). Thus, post-AAC, LV dilation was associated with a progressive decline in fractional shortening (FS), which was evident by 2 weeks (42±2% versus sham: 60±2%; P<0.0001) and continued to decline significantly at 4 and 8 weeks (Figure 1D).

Fenofibrate treatment significantly inhibited the increase in total wall thickness. This was evident at 2 weeks post-AAC (0.83±0.01 mm versus untreated AAC; P<0.001) and persisted at 4 and 8 weeks post-AAC (Figure 1A). In concert, by 2 weeks post-AAC, fenofibrate significantly reduced the increases in LV end diastolic dimension and LV end systolic dimension (3.85±0.07 mm and 2.2±0.07 mm versus untreated AAC, respectively; P<0.001; Figure 1B and 1C). In doing so, fenofibrate prevented the decrease in the percentage of FS seen at 2 (data not shown), 4, and 8 weeks with AAC (Figure 1D).

Survival in PPARα-Deficient Mice

Mice that survived the perioperative period were included in the Kaplan–Meier survival analysis as illustrated (Figure 2). Fenofibrate decreased survival in the AAC mice in comparison with the untreated AAC group (65% versus 30%; P<0.05). During the 8 weeks after surgery, there were no deaths in either sham-operated group.

BP and Cardiac Morphology in PPARα-Deficient Mice 8 Weeks Post-AAC

Systolic BP was not affected by fenofibrate. There was a trend to decrease BP in AAC mice (P not significant). In
In addition, fenofibrate treatment did not affect BP in either sham or AAC mice (Table). AAC decreased HR 8 weeks post-AAC but was no different between untreated AAC and AAC+ fenofibrate mice. Hypertrophy is seen in AAC as is evident by the increased LV heart weight/body weight ratio (Table). However, hypertrophy was increased in AAC+ fenofibrate (P<0.05 versus untreated AAC). In addition, fenofibrate also increased pulmonary congestion in AAC mice as shown by the increased wet/dry lung ratio (Table).

**Fenofibrate Effects on LV Structure and Function in PPARα-Deficient Mice**

Similar to the increased LV heart weight/body weight ratio (Table), fenofibrate increased total wall thickness in AAC (P<0.05) by echocardiography (Figure 3A). Regardless of the treatment in PPARα-deficient mice, AAC increased both LV end diastolic dimension and LV end systolic dimension. These were significant at 4 weeks and were further increased at 8 weeks (Figure 3B and 3C). AAC+ fenofibrate hearts were more dilated (Figure 3B and 3C). In concert, LV FS was progressively decreased in all of the AAC PPARα-deficient mice that were significant at 4 weeks and further decreased at 8 weeks. However, fenofibrate exacerbated the decline in LV percentage of FS in AAC at both 4 and 8 weeks (Figure 3D).

**Myocardial PPARγ and PPARβ/δ Expression in PPARα-Deficient Mice**

In sham and AAC hearts, PPARγ protein expression was not detectable by Western blot analysis regardless of treatment (data not shown). Similarly, PPARβ/δ protein expression was determined by Western blot analysis. Fenofibrate-treated AAC hearts exhibited a >9-fold increase in PPARβ/δ expression compared with untreated AAC hearts and sham hearts (14.7±4.6 versus 1.6±0.7 versus 1.7±0.6 arbitrary units (AU), respectively; Figure 4A and 4B).

**Myocardial MMP and TIMP Expression in PPARα-Deficient Mice**

The balance between MMP and TIMP expression is partially responsible for changes in the extracellular matrix. At 8 weeks post-AAC, MMP-2 protein expression was increased 39±26% (P<0.01 versus sham mice). Fenofibrate increased MMP-2 expression by 51±20% post-AAC (P<0.01 versus sham+fenofibrate). But, there was no difference between the untreated AAC and AAC+ fenofibrate groups. Similarly, TIMP-2 expression was increased post-AAC untreated (50±11%; P<0.01 versus sham) but not in AAC+ fenofibrate (22±16%; P not significant versus sham+fenofibrate; Figure 5A through 5C). Thus, the resulting MMP-2/TIMP-2 ratio, an index of net MMP activation, in PPARα-deficient mice was increased in AAC+ fenofibrate and unchanged in untreated-AAC hearts (6.9±1.0 versus 5.0±0.4; P<0.05; Figure 5G).

MMP-9 expression was not affected by AAC and fenofibrate treatment. TIMP-1 expression, its inhibitor, was increased post-AAC compared with untreated sham (11.4±4.3 versus 4.7±0.5 AU) and further increased in AAC+ fenofibrate hearts (22.8±1.9 AU). Thus, the ensuing MMP-9/TIMP-1 ratio was significantly decreased (~2.5 fold) in AAC+ fenofibrate versus untreated AAC (1.0±0.1 versus 2.7±0.8; P<0.05; Figure 5D through 5G).

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<th>Characteristics of PPARα-Deficient Mice at 8 Weeks After Sham and AAC Operation and Chronic Treatment With Fenofibrate</th>
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<td><strong>Group B</strong></td>
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LW/BW indicates the ratio of LV weight to body weight; PPARα−/−, PPARα deficient; Fen, fenofibrate. Data are represented as mean±SEM.

P<0.01 vs Sham+fenofibrate, †P<0.05 vs Sham, ‡P<0.001 vs Sham+fenofibrate.

§P<0.05 vs AAC.
Myocardial Fibrosis in PPARα-Deficient Mice

Examination of cross-sections taken from the LV showed positive trichrome staining indicative of fibrosis. Fibrosis was increased 69% in AAC+ fenofibrate hearts compared with untreated AAC hearts (Figure 6A and 6B).

In Vitro Studies

Fenofibrates Induces ERK Phosphorylation in Culture in Aldosterone-Treated ARVMs

We showed previously in ARVMs that aldosterone directly induces MMP-2 and MMP-9 activities. The mechanism

Figure 3. In vivo transthoracic echocardiography measurements performed at 4 and 8 weeks after AAC PPARα-deficient mice. A, Total wall thickness; B, LV diameter end diastole; C, LV diameter end systole; D, FS; in untreated Sham (□, n=6), Sham+Fen (■, n=6), untreated AAC (▲, n=8 to 10), and AAC+Fen (●, n=8 to 10) mice. *P<0.05 vs untreated Sham; †P<0.0 vs Sham+Fen; ‡P<0.001 vs Sham+Fen; §P<0.01 vs untreated AAC; #P<0.05 vs untreated AAC. Fen indicates fenofibrate.

Figure 4. PPARβ/δ protein expression in AAC PPARα-deficient mice. A, Fenofibrate treatment increased myocardial PPARβ/δ protein expression in AAC mice. PPARβ/δ protein expression was increased ~9 fold (†P<0.05 vs Sham+Fen). There was minimal PPARβ/δ protein expression in untreated AAC vs AAC+Fen hearts (§P<0.05). Data are mean±SEM; n=3 to 4. Fen indicates fenofibrate B, Representative Western blot of PPARβ/δ expression. There was no difference between untreated sham and Sham+Fen, thus, a representative sham was shown.
partially involves activation of the MEK/ERK1/2 pathway. We, thus, sought to explore the role of fenofibrate in aldosterone-mediated MMP expression in ARVMs. By Western blotting, treatment with aldosterone (50 nM) and fenofibrate (100 \(\mu\)mol/L) for 30 minutes increased phospho-ERK1/2 by 55\%/11006 11% and 32\%/11006 6%, respectively. ERK1/2 phosphorylation tended to increase further when aldosterone was pretreated with fenofibrate (10 \(\mu\)mol/L; \(P\) not significant versus aldosterone alone). Fenofibrate (100 \(\mu\)mol/L) for 30 minutes also increased phospho-ERK1/2, but the addition of aldosterone inhibited the increase in phospho-ERK1/2 by 44\% (Figure 7A and 7B).

**Fenofibrate Inhibits Aldosterone-Induced Increases in MMP Activity in ARVMs**

In-gel zymography of conditioned medium from ARVM, treated with aldosterone (50 nM) for 24 hours, significantly increased MMP-2 (81\%/11006 36\%) and MMP-9 (32\%/11006 5\%) activities versus control (Figure 7C through 7E). Pretreatment with fenofibrate at 10 \(\mu\)mol/L and 100 \(\mu\)mol/L significantly abolished the increase in MMP-2 activity (Figure 7D) seen with aldosterone-treatment.
Similarly, the aldosterone-induced increase in MMP-9 gelatinase activity was also significantly inhibited by both doses of fenofibrate (10 μmol/L and 100 μmol/L; Figure 7E).

**Discussion**

In this study, fenofibrate ameliorated progressive LV dilation and systolic failure seen with AAC in chronic PO WT mice. These improved effects seen with fenofibrate are because of activation of the PPARα ligand. In contrast, in a PPARα-independent manner, fenofibrate exacerbated LV dilation, systolic failure, fibrosis, and decreased survival in chronic PO PPARα-deficient mice. These PPARα-independent effects were associated with impaired matrix turnover as shown by the increased MMP-2/TIMP-2 ratio. There was also increased myocardial PPARβ/δ expression in fenofibrate-treated PO PPARα-deficient mice. The beneficial effects of fenofibrate, via activation of the PPARα ligand, inhibited aldosterone-induced phospho-ERK1/2 and MMP activity in cultured ARVM.

PPARα-deficient mice are reportedly refractory to pleotropic responses induced by PPARα ligands. Moreover, cardioprotective effects of PPARα activation during ischemia/reperfusion are abolished in PPARα-deficient mice. Similarly, myocardial fibrosis appears in an age-dependent manner in PPARα-deficient mice. We did not see evidence of cardiac fibrosis in sham PPARα-deficient mice, and this may be because of the younger age of our mice by the end of the study. Similarly, unlike the findings of Loichot et al., we do not see decreased cardiac function or HR at baseline compared with WT mice (Figures 1 and 3). Loichot et al sedated their mice for echocardiography, and, because anesthesia decreases HR, which correlates with depressed cardiac function and contractility, this may explain their adverse findings.

Nonetheless, our study aims to address the role of fenofibrates in pathological remodeling. Our data suggest that PPARα-independent actions of fenofibrate increase fibrosis and matrix turnover and are partly responsible for the adverse effects seen in the chronic PO myocardium. In the absence of PPARα, fenofibrate exerted deleterious effects, which were associated with increased MMP-2 expression and a reduction in TIMP-2 expression. The myocardial MMP-2/TIMP-2 ratio, an index of net MMP activation, was increased in fenofibrate-treated PO PPARα-deficient mice, as was the fibrosis indicating increased matrix turnover, which may contribute to the LV hypertrophy seen. The increased LV hypertrophy seen with fenofibrate treatment in PPARα-deficient mice was not associated with elevated BP. Additionally, there was increased ANP expression (data not shown), a molecular marker of hypertrophy, in fenofibrate-treated PO PPARα-deficient mice. Fenofibrate had no affect on sham PPARα-deficient mice, suggesting that these negative effects may be apparent only during conditions of "stress," for example, such as during renin–angiotensin–aldosterone system activation with increased aldosterone or PO. The adverse effects (cardiac remodeling and fibrosis) of fenofibrate were not because of a lowering of tail-cuff BP (because there is no difference between treated or untreated PO PPARα-deficient mice). There was evidence of “clinical” heart failure in the mice, as demonstrated by a trend to decreased systolic BP in both of the AAC (PO) groups, the slight decrease in HR, and the increased pulmonary congestion (ie, wet/dry lung ratio).

Studies have demonstrated that MMP-2 and MMP-9 are augmented in dilated failing hearts and involved in the development and progression of myocardial remodeling. Similarly, MMP-2 and MMP-9 are increased during ischemia/reperfusion and are attenuated by PPARα agonists. Selective disruption of the MMP-2 gene amelioriated myocyte...
Figure 7. Fenofibrate induces ERK signaling in aldosterone-treated ARVMs. A, Treatment of ARVM with aldosterone (50 nM) and fenofibrate (10 μmol/L) increased phospho-ERK1/2 by 55±11% and 32±6%, respectively (#P<0.01 vs control). Phospho-ERK1/2 tended to increase further when fenofibrate (10 μmol/L) was added to the aldosterone (P not significant vs aldosterone). Fenofibrate (100 μmol/L) for 30 minutes also increased phospho-ERK1/2 (*P<0.05 vs control), but the addition of aldosterone inhibited the increase in ERK1/2 phosphorylation by 44% (‡P<0.01 vs fenofibrate, 100 μmol/L; §P<0.05 vs aldosterone). Data are normalized to total ERK. B, Representative Western blot of ERK signaling. Data are mean±SEM. Fenofibrate abrogates aldosterone-stimulated MMP activity in ARVM. C, Representative gelatin zymogram of MMP activity in conditioned medium taken from untreated LV myocytes and those treated with 50 nM aldosterone (Aldo); 10 μmol/L Fen; Fen (10 μmol/L)/Aldo; 100 μmol/L Fen; Fen (100 μmol/L)/Aldo (10^5 total cells; n=6). MMP gelatinolytic activity was observed between 100 and 50 kDa, which is consistent with MMP-2 and MMP-9. MMP gelatinase activity is summarized in D and E. Fen indicates fenofibrate. D, MMP-2 gelatinase activity was localized to ~70 kDa. MMP-2 gelatinase activity in LV myocyte-conditioned medium was increased by 81±36% after treatment with aldosterone (*P<0.05 vs control). Fenofibrate at both 10 μmol/L and 100 μmol/L doses inhibited the increase in MMP-2 activity by ~88±14% and ~120±6%, respectively, seen with aldosterone (‡P<0.01 vs aldosterone). E, MMP-9 gelatinase activity was localized to ~90 kDa. MMP-9 gelatinase activity was increased 32±5% after treatment with aldosterone (#P<0.01 vs control). Fenofibrate at both 10 μmol/L and 100 μmol/L doses inhibited the increase in MMP-9 activity 38±3% and 64±12%, respectively, seen with aldosterone (§P<0.01 vs aldosterone). Data are mean±SEM.
hypertrophy and interstitial fibrosis in PO mice. Thus, our data support the evidence that MMP-2 is involved in mediating PO-induced ventricular hypertrophy. Although MMP-2 was increased in untreated PO PPARα-deficient mice, it was further increased with fenofibrate treatment. TIMP-2 was increased in untreated AAC, but the expression of this inhibitor of MMP-2, in fenofibrate-treated AAC, was not as distinct. Although MMP-9 expression was not increased in fenofibrate-treated AAC, its inhibitor TIMP-1 possibly compensated for the lack of TIMP-2 expression by increased TIMP-1 expression. Similarly, fenofibrate may increase TIMP-1 expression to increase MMP-2 expression (because TIMPs participate in the process of MMP activation). Although we could not test for a cause or an effect in this in vivo model, the lack of an increase in the MMP-9/TIMP-1 ratio in AAC fenofibrate mice may contribute to the adverse effects of fenofibrate on cardiac function and survival. Thus, PPARα may have a protective role in preventing fenofibrate-induced exacerbation of hypertrophy in this model by its effect on MMP and TIMP interactions. Fenofibrate is a weak activator of PPARβ/δ and in other cell systems activates other pathways that are not PPAR dependent, such as non–receptor-mediated (non-genomic) effects. There are other possible hypotheses for an unfavorable outcome in fenofibrate-treated PO PPARα-deficient mice in addition to increasing fibrosis, mortality, and activating MMPs. These non-PPARα-mediated effects include proapoptosis by inhibiting Akt phosphorylation and proapoptotic gene activation resulting in the progression to heart failure. In addition, there may be a precedent for significant species variation in PPAR-mediated responses, but they may also be related to the experimental model used to induce cardiac remodeling, for example, rats with myocardial infarction, pigs with ischemia/reperfusion, and humans with diabetes.

We also measured other isoforms of PPAR ligands in this whole body deletion of PPARα. There was negligible myocardial expression of PPARγ. Interestingly, myocardial expression of PPARβ/δ was significantly increased in fenofibrate-treated PO PPARα-deficient mice. The role of PPARβ/δ is unclear in cardiac remodeling. It has been suggested that PPARβ/δ agonists inhibit cardiac hypertrophy. Our data do not demonstrate a cause and an effect; increased PPARβ/δ expression in fenofibrate-treated PO PPARα-deficient mice is associated with adverse cardiac remodeling and requires further investigation. It is more likely that the adverse effects of fenofibrate would be even more marked in the absence of PPARβ/δ upregulation. Thus, PPARβ/δ expression may merely be a marker of comparably worse LV function and adverse remodeling. PPARβ/δ expression was measured in fenofibrate-treated WT PO mice with chronic remodeling, that is, in the presence of PPARα ligands; there was no upregulation in PPARβ/δ expression (data not shown).

Similar to Dahl salt-sensitive rats and deoxycorticosterone acetate-salt hypertensive rats, fenofibrate, in our study, exerts beneficial effects on cardiac remodeling via PPARα activation in WT mice. The beneficial effect seen in our study is a decrease in wall thickness, a reduction in ventricular dilation, and an improvement in FS. We have shown that inflammatory cytokines and reactive oxygen species regulate myocardial MMP and we now demonstrate that fenofibrate regulates the extracellular matrix in cardiac remodeling. The benefits of fenofibrates (by inhibiting aldosterone-induced increases in MMP activity) in vitro are partly because of PPARα-dependent activation by fenofibrate, and there was no increased PPARβ/δ or PPARγ expression in ARVMs treated with fenofibrate (data not shown). The mechanisms of the improved chronic cardiac remodeling with fenofibrate (unrelated to their lipid-lowering actions) have been associated with effects on the vasculature, inhibition of proinflammatory and redox-sensitive transcription factors, and on cell infiltration and MMP-9 inhibition.

**Perspectives**

Fenofibrates are beneficial in progressive PO-induced cardiac remodeling, likely because of the presence of PPARα. Our study demonstrates, in cardiac remodeling, that the benefits of fenofibrate are partly because of aldosterone-stimulated inhibition of phospho-ERK and MMP. The adverse effects of fenofibrate (present only when PPARα is absent) are because of activation of MMP, increased fibrosis, left ventricular hypertrophy, and increased mortality. These findings indicate that, with PPARα deficiency, fenofibrates exert deleterious pleiotropic actions in the failing myocardium.

Interestingly, in human studies, several polymorphisms of the PPARα locus have been described. The V162 allele is associated with alterations in lipids and decreased cardiovascular events in diabetics. The effect of PPARα agonists with this allele is variable and requires further investigation. Because current heart failure therapies are directed at the inhibition of neurohormonal pathways, these results may provide a basis for future strategies to prevent or reverse cardiac remodeling in PO hypertrophy. However, despite their positive effects on cardiac physiology in vivo, the therapeutic benefit for fibrates in human heart failure requires longer-term studies to monitor their effects on cardiac function. Moreover, closer observation may be necessary in humans undergoing chronic fibrate therapy.

**Sources of Funding**

This work was supported in part by National Institutes of Health grants HL004423 (F.S.) and HL079099 (F.S.).

**Disclosures**

None.

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Hypertension. 2007;49:1084-1094; originally published online March 12, 2007;
doi: 10.1161/HYPERTENSIONAHA.107.086926
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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