Effects of Fenofibrate on Cardiac Remodeling in Aldosterone-Induced Hypertension

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Abstract—Hypertension and cardiac remodeling are associated with myocardial fibrosis, left ventricular (LV) hypertrophy, and diastolic heart failure. Fenofibrate suppresses aldosterone-mediated increases in myocyte matrix metalloproteinase activity and extracellular signal–regulated kinase phosphorylation. It is unknown whether the peroxisome proliferator–activated receptor-α agonist, fenofibrate, improves cardiac remodeling in a model of aldosterone-induced hypertension and LV hypertrophy. Twelve-week-old uninephrectomized FVB mice received 1% NaCl drinking water. Miniosmotic pumps delivered saline or aldosterone for 4 weeks. Mice were either untreated (n = 14) or treated with fenofibrate 100 mg/kg per day (n = 12) for 1 week before and 4 weeks after surgery. Aldosterone increased systolic blood pressure in untreated mice versus saline-untreated mice (134±3 versus 91±3 mm Hg; P<0.01). This was unaffected by fenofibrate (131±3 mm Hg). Aldosterone increased LV end-diastolic and end-systolic dimensions, which were significantly attenuated by fenofibrate (3.8±0.1 versus 3.5±0.1 mm, and 1.5±0.1 versus 1.15±0.1 mm, respectively). Fenofibrate also decreased aldosterone-induced LV hypertrophy (LV weight/body weight, 4.1±0.2 versus 4.6±0.1 mg/g) and improved percent LV fractional shortening (67±7% versus 60±2%). Additionally, fenofibrate ameliorated the increased matrix metalloproteinase-2/tissue inhibitors of metalloproteinase-2 ratio and fibrosis seen in aldosterone-untreated hearts (P<0.05 for both). Furthermore, in aldosterone-untreated hearts, fenofibrate decreased transforming growth factor-β, collagen type III (P<0.05 for both), and collagen type I (P<0.01) protein expression. Conversely fenofibrate increased peroxisome proliferator–activated receptor-α, peroxisome proliferator–activated receptor-γ coactivator-1α expression, and acetyl coenzyme A carboxylase phosphorylation (P<0.05 for all) in aldosterone-infused hearts; uncoupling protein-3 and medium-chain acyl coenzyme A dehydrogenase protein expression decreased with fenofibrate (P<0.05 and P<0.01, respectively, versus aldosterone-infused), suggesting that improved myocardial remodeling is independent of fatty acid oxidation. Thus, fenofibrate improved aldosterone-induced LV hypertrophy independently of an effect on blood pressure with decreased fibrosis and altered extracellular matrix.

Key Words: fibrosis ■ aldosterone ■ hypertension ■ cardiac remodeling ■ matrix metalloproteinases ■ peroxisome proliferator–activated receptor-α

Diastolic heart failure (HF) refers to “HF with preserved left ventricular (LV) ejection fraction”1–3 and is observed in almost half of the patients who present with clinical HF.2 Hypertension, a significant risk factor for diastolic dysfunction and HF,4 is frequently associated with cardiac remodeling and LV hypertrophy (LVH).5 Similarly, LVH and diastolic dysfunction are associated with matrix metalloproteinase (MMP) activation that favors decreased extracellular matrix degradation and increased interstitial collagen,6 thus perpetuating fibrosis. Tissue inhibitors of metalloproteinases (TIMPs) may predict HF in chronic hypertension.7 Likewise, aldosterone antagonists (used in chronic HF and in hypertension) exert positive effects by downregulating MMP activity.8–11 Despite the increasing incidence of diastolic HF and elevated mortality rates,12 there remains a paucity of therapies that target these potential underlying mechanism(s).

Fenofibrate, a peroxisome proliferator–activated receptor-α (PPAR-α) agonist, is used clinically for the treatment of dyslipidemias. Intriguingly, fibrates are being considered as possible therapeutic agents for the treatment of cardiac remodeling.13 The myocardial effects of fenofibrate are independent of its lipid-lowering actions and are partly due to PPAR-α–mediated suppression of inflammatory transcription factors (eg, nuclear factor-κB and activating protein-1)14–16 and inhibition of macrophage recruitment.17 Inhibition of nuclear factor-κB with fenofibrate in hypertension prevents the development of diastolic dysfunction.18 We previously demonstrated in isolated adult cardiomyocytes that fenofibrate...
inhibits MMP activity and aldosterone-stimulated increases in extracellular signal–regulated kinase phosphorylation.

In the present study, we used a murine model of aldosterone-induced hypertension and diastolic HF to test the hypothesis that fenofibrate exerts beneficial effects on myocardial structure, function, and matrix.

Materials and Methods

Animals

Study protocols were reviewed and approved by the institutional animal care and use committee of the Boston University School of Medicine. In brief, 12-week-old FVB mice (Charles River, Wilmington, Mass) were maintained on a 12-hour light/dark cycle in a temperature-controlled (19°C to 21°C) room and were fed standard rodent chow ad libitum.

Aldosterone Infusion

Uninephrectomized mice (27 to 30 g) received an osmotic minipump (Alzet, Durect Corp) that delivered a continuous infusion of either saline or d-aldosterone (0.15 μg/h; Sigma-Aldrich) for 4 weeks.20,21 Additionally, all mice were maintained on 1% NaCl drinking water.

Treatment

Thirty mice were randomly assigned to either regular chow or chow containing fenofibrate (100 mg/kg body weight [BW] per day) for 1 week before and 4 weeks after surgery. The dose of fenofibrate has been used by others22,23 and has been shown to be nontoxic. The 4 groups examined were as follows: saline-untreated, salinedifenofibrate, aldosterone-untreated, and aldosterone-fenofibrate.

Physiologic Measurements

After 4 weeks of saline or aldosterone infusion, heart rate and systolic blood pressure (BP) were measured with a noninvasive tail-cuff system (BP-2000, VisiTech) as previously described.19,24 Transthoracic echocardiography was performed in conscious mice 4 weeks after surgery. Echocardiography was performed as previously described16 with an Acuson Sequoia C-256 echocardiograph machine and a 15-MHz probe.

Organ Weights and Tissue Analysis

After 4 weeks, mice were euthanized, BW was measured, and the hearts were either (1) arrested in diastole by KCl (30 mmol/L), weighed, perfused with 10% buffered formalin, and sliced horizontally for histology or (2) snap-frozen in LN2. To measure fibrosis, trichrome-stained sections (5 μm) were visualized by light microscopy, and the entire section was quantified with the use of Bioquant Image analysis software. Additionally, frozen LV transverse sections were examined for intramyocardial lipid accumulation by staining with oil red O. The wet-to-dry weights of the lungs were determined, and the ratio was taken as an index of pulmonary congestion and clinical HF.18

Immunoblot Analysis

Aliquots of tissue protein (30 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech). Membranes were probed with antibodies specific to MMP-9, MMP-2, TIMP-1, TIMP-2 (Chemicon), medium-chain acyl-coenzyme A dehydrogenase (MCAD), uncoupling protein-3 (UCP3, Cayman), transforming growth factor (TGF)-β (R&D Systems), collagens I and III (Rockland), pan–AMP-activated protein kinase-α (AMPK, Cell Signaling), phosphoacetyl coenzyme A carboxylase (Ser-79, Upstate), PPAR-γ coactivator-1α (PGC-1α, Chemicon), or actin (Sigma) for 1 hour at room temperature or, specific to phospho-AMPK (Thr-172, Cell Signaling) or PPAR-α (Santa Cruz Biotechnology), overnight at 4°C. Bound antibodies were detected with the appropriate horseradish peroxidase–linked whole secondary antibodies. All blots were normalized against Coomassie brilliant blue staining of the gels (Sigma-Aldrich). Protein immunoblots were visualized by enhanced chemiluminescence, and bands were quantified by scanning densitometry (Bio-Rad).

Statistical Analysis

Results are presented as mean±SEM. Differences between mice with and without fenofibrate treatment were tested with Student t tests or 2-way ANOVAs followed by Student-Newman-Keuls post hoc tests, which were used for establishing significant differences among groups. A value of P<0.05 was considered statistically significant.

Results

Aldosterone- and Fenofibrate-Mediated Effects on Cardiac Physiology and Morphology

Four weeks of aldosterone infusion in uninephrectomized mice significantly increased systolic BP in both untreated (134±3 mm Hg) and fenofibrate-treated (131±3 mm Hg) mice versus saline-infused controls (P<0.01 for both; the Table). Fenofibrate had no effect on BP in either saline-unreated or aldosterone-infused groups. Heart rate was not affected by aldosterone infusion alone; however, fenofibrate modestly increased heart rate in aldosterone-infused mice. Additionally, during the 4 weeks after surgery, there were no deaths in either sham or aldosterone groups.

An increase in BW was observed in aldosterone-infused mice, but this was unaffected by fenofibrate treatment (P=NS). As expected, aldosterone caused cardiac hypertrophy, as reflected by an increased LV weight–to–BW (LV/BW) ratio (21% increase, P<0.01 versus saline-untreated). As shown in the Table, fenofibrate significantly reduced aldosterone-mediated cardiac hypertrophy and the LV/BW ratio (4.6±0.1 versus 4.1±0.2 mg/g). Aldosterone infusion also caused significant pulmonary venous congestion, as indicated by the increased wet-to-dry lung weight ratio. This wet-to-dry ratio, an indicator of pulmonary congestion and clinical HF,25 was unaffected by fenofibrate treatment. Thus, fenofibrate ameliorated aldosterone-induced LVH but had no effect on systolic BP or pulmonary venous congestion.
Aldosterone- and Fenofibrate-Mediated Effects on Cardiac Structure and Systolic Function

Modest increases in both LV end-diastolic diameter (+36%, \(P<0.01\)) and LV end-systolic diameter (+27%, \(P<0.01\)) were elicited by aldosterone versus saline-untreated (Figures 1A and 1B). The increase in LV end-systolic diameter with aldosterone infusion was completely abrogated by fenofibrate treatment, and the increase in LV end-diastolic diameter was partially suppressed \((P<0.05\) versus aldosterone-untreated; Figures 1A and 1B). Collectively, these effects of fenofibrate on aldosterone-induced changes in LV systolic function resulted in a relative (but not significant) increase in percent fractional shortening (Figure 1C). Thus, aldosterone-mediated HF occurred in the absence of a decrease in fractional shortening, consistent with a model of diastolic HF (pulmonary venous congestion and “normal” LV fractional shortening). Similar to the LV/BW ratio (the Table), the interventricular septal wall thickness was attenuated with fenofibrate in the aldosterone-infused mice \((P<0.05\) versus aldosterone-untreated; Figure 1D). Thus, fenofibrate ameliorated aldosterone-induced LV dilation and hypertrophy but had no effect LV systolic function.

Aldosterone- and Fenofibrate-Mediated Effects on Myocardial Fibrosis

LV cross sections from aldosterone-untreated mice had marked positive trichrome staining, indicative of fibrosis (Figure 2A). In aldosterone-fenofibrate mice, this was significantly less evident, consistent with decreased fibrosis (41\% ± 3\%, \(P<0.05\) versus aldosterone-untreated; Figure 2B).

To examine potential mechanisms whereby fenofibrate may exert beneficial effects on matrix turnover and fibrosis, we examined MMP and TIMP protein expression (Figure 3). In the presence and absence of fenofibrate, aldosterone significantly increased MMP-2 protein expression relative to saline-untreated mice \((P<0.01\) and \(P<0.05\), respectively). The magnitude of aldosterone-induced MMP-2 expression (Figure 3A) was decreased 25\% ± 1\% in aldosterone-fenofibrate versus aldosterone-untreated mice \((P<0.05)\). TIMP-2 expression was increased \(\approx 6\)-fold with aldosterone \((P<0.01\) versus saline-untreated) or an additional 30\% ± 7\% with fenofibrate \((P<0.05\) versus aldosterone-untreated; Figure 3B). The resulting MMP-2/TIMP-2 ratio, an index of net MMP activation, was significantly decreased in aldosterone-fenofibrate hearts \((P<0.05\) versus aldosterone-untreated; Figure 3C).

Myocardial MMP-9 and TIMP-1 expression was increased in aldosterone-untreated hearts \((P<0.01\) and \(P<0.05\), respectively, versus saline-untreated) but remained unaffected by fenofibrate treatment (Figures 4A and 4B). Thus, fenofibrate in aldosterone-untreated mice had no effect on the MMP-9/TIMP-1 ratio (Figure 4C).

Because TGF-\(\beta\) is an important regulator of MMP expression and overexpression of TGF-\(\beta\) elevates type III collagen expression in some cell types, we sought to determine whether regulators of the extracellular matrix were altered by fenofibrate (Figures 5A through 5C). Fenofibrate decreased myocardial protein expression of TGF-\(\beta\) by 3.4-fold \((P<0.05)\), type I collagen I by 3-fold \((P<0.01)\), and type III collagen by 1.5-fold \((P<0.05)\) versus aldosterone-untreated hearts. Thus, fenofibrate inhibited aldosterone-induced fibrosis and regulators of the extracellular matrix in vivo.

Aldosterone- and Fenofibrate-Mediated Effects on Myocardial Lipid Deposition

For LV myocardial tissue, longitudinal and cross sections were stained with oil red O to determine whether aldosterone-mediated cardiac remodeling was associated with lipid accumulation. Myocardial tissue sections from aldosterone-untreated mice demonstrated positive punctuate staining. As illustrated in Figure 6A, this staining was notably absent in sections from fenofibrate-treated mice and comparable with those of saline-untreated mice.

We also sought to determine whether differences in LV lipid accumulation occurred in parallel with, or affected
mediators of, fatty acid oxidation and classic PPAR-α targets with fenofibrate. We thus examined the protein expression of UCP3, MCAD, (Figures 6B and 6C), PPAR-α, PGC-1α, and the phosphorylation of acetyl coenzyme A carboxylase and AMPK (Figures 6D and 6E). Fenofibrate significantly increased the expression of PPAR-α and PGC-1α, mediators of mitochondrial biogenesis, and the phosphorylation of acetyl coenzyme A carboxylase, but not of AMPK. However, UCP3 expression tended to decrease versus saline-untreated mice, and fenofibrate further decreased UCP3 protein expression (P < 0.05 versus aldosterone-untreated). Similarly, MCAD expression, another enzyme in the fatty acid β-oxidation pathway, was measured and was similarly reduced in aldosterone-fenofibrate mice (P < 0.01 versus aldosterone-untreated; Figure 6C). Thus, fenofibrate decreased protein expression of enzymes in the oxidation pathway of long-chain fatty acids in aldosterone-mediated cardiac remodeling.

**Discussion**

In this study, after 4 weeks of aldosterone infusion, there was a significant increase in systolic BP, pronounced cardiac hypertrophy, and preserved LV systolic function. Additionally, concomitant treatment with fenofibrate had no effect on BP yet ameliorated cardiac hypertrophy. Aldosterone administration was also associated with marked fibrosis and some lipid deposition in the myocardium. These deleterious effects were markedly attenuated by fenofibrate treatment. Reduced fibrosis was associated with fenofibrate-mediated changes in the expression of MMPs and TIMPs, and decreased lipid accumulation occurred in conjunction with the reduction in cardiac hypertrophy. The decreased lipid accumulation was associated with decreased expression of the PPAR-α targets, UCP3 and MCAD.

Diastolic HF is distinguished from systolic HF by a preserved LV ejection fraction. Thus, in response to aldosterone infusion, we observed marked cardiac hypertrophy, pulmonary venous congestion, and hypertension in the presence of preserved fractional shortening. Although we did not measure LV end-diastolic pressure, it is likely that the increased pulmonary congestion reflects increased LV end-diastolic pressure,1–3 a hallmark of diastolic dysfunction. In our study, there was no therapeutic effect of fenofibrate on the wet-dry weight ratio; however, Ogata et al26 used this agent for 5 weeks and demonstrated improved diastolic function as measured by LV end-diastolic pressure,26 possibly by improving the vasculature and active relaxation of the myocardium.1,27 Whether improved diastolic function in response to fenofibrate occurred in our model and whether this effect may ameliorate venous congestion remain to be determined. Hypertension is a significant risk factor for diastolic dysfunction. Consistent with prior reports,1,18,28 we did not observe an effect of fenofibrate on BP. However, other PPAR-α agonists (ie, docosahexaenoic acid and clofibrate) have decreased BP in other models of hypertension, possibly

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by inhibiting NADPH oxidase–induced oxidative stress and inflammation in the vascular wall and reducing endothelin-B receptor blockade in the kidney. We previously showed that aldosterone increases myocyte MMP activity and extracellular signal–regulated kinase phosphorylation and may be inhibited by fenofibrate. Similar to other in vivo models of HF (ie, mice subjected to pressure overload or angiotensin II infusions), aldosterone administration induces a proinflammatory and a profibrotic phenotype.10 In our study, aldosterone-induced LVH was associated with an increased MMP-2/TIMP-2 ratio. The increased MMP activity may contribute to ventricular remodeling and LVH by impairing the integrity of the interstitial matrix.22,33 aldosterone-mediated increases in the MMP-2/TIMP-2 ratio was associated with significant interstitial fibrosis, which was attenuated by fenofibrate. Fenofibrate may exert PPAR- independent effects by increasing MMP-2 and decreasing TIMP-2 expression,18 resulting in LVH and fibrosis. Similarly, the MMP-2 gene plays a critical role in myocyte hypertrophy,36 pressure overload–induced LVH,10,31 and in the promotion of interstitial fibrosis. Thus, its inhibition may contribute to the decrease in LVH.22

TGF-β suppresses proteolytic activity by increasing TIMP, MMP-2 and MMP-9 expression.37 Similarly, TGF-β is implicated in cardiac myocyte hypertrophy38 and inhibits MMP expression by sequestering unbound activating protein-1.39 TGF-β increases type I and type III collagen synthesis40 and with aldosterone infusion, fenofibrate likely inhibits MMP-2 by inhibiting TGF-β.28,41,42 Therefore, fenofibrate mediates inhibitory effects on TGF-β and types I and III collagen and therefore inhibits MMP-2. This promotes normalization of collagen turnover and contributes, in part, to the improved LV structure and maintenance of normal LV function seen with fenofibrate. These findings complement previous reports that fenofibrate inhibits myocardial fibrosis and inflammation by inhibiting p38 nuclear factor-κB signaling pathways,44 suppressing prohypertrophic and redox-regulated transcription factors,28,31 cardiac endothelin-1 production,26,45,46 and inhibiting MMP-9. Additionally, both PPAR-α and PPAR-γ agonists inhibit TGF-β signaling by interacting with Smad transcription factors47–49 and although speculative, may constitute a mechanism for PPAR-α–mediated inhibition of TGF-β–regulated gene expression in aldosterone-induced fibrosis.

PPAR-α is regulated during development, disease, and cardiac metabolism. During acute pressure-overload HF, PPAR-α is diminished, fatty acid oxidation is reduced, and lipids accumulate in myocytes. Interestingly, fenofibrate in pressure overload has been observed to both worsen and improve heart function, depending on the duration of HF. In our study, fenofibrate eliminated aldosterone-infused myocardial lipid accumulation. The relation between lipid accumulation and fibrosis is unclear, but an interplay between tissue repair (ie, TGF-β signaling) and energy metabolism as an adaptive response to injury or stress may exist.48 Similarly, overexpressing acyl coenzyme A synthase increases myocyte triacylglycerol content and results in myofiber disorganiza-
tion, interstitial fibrosis, and LV dysfunction.52 Others have suggested a causal link between lipid accumulation and cardiac dysfunction,53,54 with myocardial lipid accumulation resulting from either an overexpression or underexpression of fatty acid enzymes.53 Interestingly, we observed decreased myocardial lipid deposition with fenofibrate and increased myocardial PPAR-α, PGC-1α, and phosphoacetyl coenzyme A carboxylase protein expression. However, UCP3 and MCAD protein expression, key mediators of fatty acid oxidation, were decreased. Our study was not designed to establish a cause or effect, nor did we measure metabolically regulated gene expression or fatty acid oxidation rates.55 Dissociation between metabolic genes and protein expression has been shown to exist.55 Another reason for our findings may be that, similar to antioxidant enzymes in HF,56 decreased UCP3 and MCAD protein expression reflects decreased translation or posttranslational modification of metabolic enzymes.57,58 Additionally, our findings may differ because of the model of HF (diastolic versus systolic), method of inducing HF (aldosterone infusion versus pacing induced59), and animal species (mice versus dogs). Lastly, the light/dark cycle (circadian clock) has been suggested as a mechanism that regulates myocardial metabolic genes ie, alterations in gene expression depending on the light/dark cycle.60

Fenofibrates are PPAR-α ligands that activate both PPAR-β/δ and PPAR-α targets.61 PPAR-β/δ and PPAR-α regulate similar genes in cardiac remodeling64 and share comparable functions in cardiac fatty acid oxidation.62 The role of PPAR-β/δ is unclear in cardiac remodeling. On one hand, PPAR-β/δ agonists inhibit cardiac hypertrophy15 and on the other hand, a PPAR-β/δ deficiency promotes cardiomyopathy, LV dysfunction, and lipid accumulation.63 PPAR-α and PPAR-β/δ regulate UCP3 gene expression in skeletal muscle and the heart and mediate UCP3 regulation by free fatty acids. When the heart is “stressed” by free fatty acid overload, as in HF, UCP3 may favor free fatty acid and decreased excessive reactive oxygen species production. Thus, reduced cardiac hypertrophy and lipid accumulation with fenofibrate are not due to increased fatty acid oxidation protein expression in our model.
Perspectives

Fenofibrate abrogated aldosterone-induced cardiac hypertrophy, independent of alterations in BP and pulmonary venous congestion. It was associated with effects on the expression of key factors involved in matrix turnover, resulting in decreased fibrosis and lipid accumulation in the myocardium. Although speculative, coordinated inhibition of myocardial fibrosis and effects on metabolism warrant further study and may be beneficial in hypertension-mediated diastolic dysfunction.

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Disclosures

None.

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