Fenofibrate inhibits aldosterone-induced apoptosis in adult rat ventricular myocytes via stress-activated kinase-dependent mechanisms

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Fenofibrate inhibits aldosterone-induced apoptosis in adult rat ventricular myocytes via stress-activated kinase-dependent mechanisms. Am J Physiol Heart Circ Physiol 296: H1983–H1993, 2009. First published April 24, 2009; doi:10.1152/ajpheart.00002.2009.—Aldosterone induces extracellular signal-regulated kinase (ERK)-dependent cardiac remodeling. Fenofibrate improves cardiac remodeling in adult rat ventricular myocytes (ARVM) partly via inhibition of aldosterone-induced ERK1/2 phosphorylation and inhibition of matrix metalloproteinases. We sought to determine whether aldosterone caused apoptosis in cultured ARVM and whether fenofibrate ameliorated the apoptosis. Aldosterone (1 μM) induced apoptosis by increasing terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)-positive nuclei in ARVM. Spironolactone (100 nM), an aldosterone receptor antagonist, but not RU-486, a glucocorticoid receptor, inhibited aldosterone-mediated apoptosis, indicating that the mineralocorticoid receptor (MR) plays a role. SP-600125 (3 μM)—a selective inhibitor of c-Jun NH2-terminal kinase (JNK)—inhibited aldosterone-induced apoptosis in ARVM. Although aldosterone increased the expression of both stress-activated protein kinases, pretreatment with fenofibrate (10 μM) decreased aldosterone-mediated apoptosis by inhibiting only JNK phosphorylation and the aldosterone-induced increases in Bax, p53, and cleaved caspase-3 and decreases in Bcl-2 protein expression in ARVM. In vivo studies demonstrated that chronic fenofibrate (100 mg·kg body wt −1 ·day −1) inhibited myocardial Bax and increased Bcl-2 expression in aldosterone-induced cardiac hypertrophy. Similarly, eplerenone, a selective MR inhibitor, used in chronic pressure-overload ascending aortic constriction inhibited myocardial Bax expression but had no effect on Bcl-2 expression. Therefore, involvement of JNK MAPK-dependent mitochondrial death pathway mediates ARVM aldosterone-induced apoptosis and is inhibited by fenofibrate, a peroxisome proliferator-activated receptor (PPARα) ligand. Fenofibrate mediates beneficial effects in cardiac remodeling by inhibiting programmed cell death and the stress-activated kinases.

cardiomycocytes; stress-activated kinases

ALDOSTERONE CAUSES programmed cell death in cardiomyocytes in ischemia-reperfusion injury, myocardial infarction, and heart failure (41). However, the mechanisms of aldosterone-mediated stress signaling pathways induced in cardiomyocytes are not yet fully elucidated. Aldosterone mediates extracellular signal-regulated kinase (ERK)-dependent cardiac remodeling in cardiomyocytes (10, 19, 36). In neonatal rat ventricular cardiomyocytes (NRVM), activation of calcineurin-dependent mitochondrial death signaling pathways (24) has been suggested as a mechanism of aldosterone-induced apoptosis. Cardiomyocyte apoptosis is regulated via ERK1/2, p38 kinase, and c-Jun NH2-terminal kinase (JNK) pathways in response to a wide range of cardiac stressors (1).

Aldosterone binds to mineralocorticoid receptors (MRs), which are present on cardiomyocytes (36). Spironolactone, a nonselective MR antagonist, and eplerenone, a selective MR antagonist, improved survival and myocardial remodeling in human and experimental models of cardiac hypertrophy and heart failure (17, 31–33, 43). Inhibiting the binding of aldosterone to the MR prevents downstream signaling events such as ERK1/2 (36) and p38 phosphorylation (20).

Similarly, peroxisome proliferator-activated receptor (PPARα) agonists are being considered as therapeutic agents to modulate cardiac remodeling (2, 12, 23) alone or in conjunction with other agents (4, 5). Fenofibrate, a PPARα agonist and a fibrate, is used clinically to treat hyperlipidemias. Fenofibrate improves adverse cardiac remodeling partly by inhibiting aldosterone-stimulated ERK1/2 phosphorylation and inhibiting matrix metalloproteinases (MMPs) (10).

These findings thus led to our hypothesis that aldosterone may induce apoptosis in cardiomyocytes by differential activation of distinct kinases. We sought to explore the mechanism(s) of aldosterone-induced apoptosis in cultured adult rat ventricular myocytes (ARVM) and to test whether fenofibrate could reduce apoptosis by affecting other regulators of stress-induced apoptosis, such as the anti- and proapoptotic Bcl-2 family members Bcl-2/Bax.

MATERIALS AND METHODS

Isolation and Treatment of Adult Rat Cardiac Myocytes

As described previously (36), ARVM (90–95% purity) were isolated from the hearts of adult Sprague-Dawley rats, plated at a nonconfluent density of 30–50 cells/mm2 on plastic culture dishes (Fisher) precoated with laminin (1 μg/cm2, Invitrogen), and maintained in ACCT medium (DMEM, 2 mg/ml BSA, 2 mmol/l L-carnitine, 5 mmol/l creatinine, 5 mmol/l taurine, 100 IU/ml penicillin, and 10 g/ml streptomycin) for 16 h before drug treatment. ARVM were treated with aldosterone (1 μM, Sigma) for 30 min for signaling and 24 h for terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL). Fenofibrate (10 μM, Sigma) was added 30 min before aldosterone. In some experiments spironolactone (100 nM, Sigma), RU-486 (1 μM, Sigma), SB-203580 (3 μM, Calbiochem), SP-600125 (2 μM, Calbiochem), actinomycin D (5 μg/ml, Sigma), and cycloheximide (10 μg/ml, Sigma) were also added 30 min before aldosterone.

Detection of Stress Signaling Pathway

ARVM or homogenized tissue was collected in lysis buffer. Protein concentration was determined by Bradford assay (Bio-Rad). Proteins were probed with anti-phospho-p38 (Cell Signaling), anti-p38 (Cell Signaling), anti-phospho-JNK (Santa Cruz), anti-JNK (Santa Cruz), anti-Bax (Cell Signaling), anti-Bcl-2 (Santa Cruz), anti-p53 (Santa
Cruz), anti-cleaved caspase-3 (Cell Signaling Technology), and anti-GAPDH (Santa Cruz) antibodies. Chemiluminescence was quantified by densitometry (Molecular Analyst, Bio-Rad). All blots were normalized with Coomassie brilliant blue staining of the gels (Sigma-Aldrich).

**Myocyte Viability and Apoptosis**

Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer’s instructions (American Type Culture Collection) and calculated as a percentage of the control group. Myocyte apoptosis was assessed by TUNEL as previously described (17) according to the manufacturer’s protocol (Roche). The percentage of TUNEL-positive nuclei relative to total nuclei was determined in a blinded manner by counting 200–300 cells in 20 randomly chosen fields per coverslip for each experiment. Nuclei were counterstained with Hoechst 33342 (10 μg/ml for 10 min at room temperature) or DAPI staining (Invitrogen). Nucleosome fragmentation was assessed by enzyme-linked immunosorbent assay using the Cell Death Detection kit (Roche Applied Science).

**Animals**

Male FVB mice (Charles River, Wilmington, MA) were maintained on a 12:12-h light-dark cycle in a temperature-controlled (19–21°C) room and underwent either aldosterone infusion with/without fenofibrate therapy (model A) (19) or ascending aortic constriction (AAC) surgery with/without eplerenone therapy (model B) (10). The Institutional Animal Care and Use Committee at Boston University School of Medicine approved all study procedures related to handling and surgery of the mice.

**Model A: aldosterone infusion.** Uninephrectomized mice received an osmotic minipump (Alzet, Durect) that delivered a continuous infusion of either saline or $d$-aldosterone (0.15 μg/h; Sigma-Aldrich) for 4 wk. All mice were given 1% NaCl in the drinking water. Mice were randomly assigned to regular chow or chow containing fenofibrate (100 mg/kg body wt/1 day) for 1 wk before and 4 wk after

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**Fig. 1.** A: aldosterone stimulated deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)-positive nuclei in adult rat ventricular myocytes (ARVM) via the mineralocorticoid receptor. ARVM were incubated with 1 μM aldosterone (Aldo) for 24 h, and apoptosis was determined with the TUNEL assay. TUNEL-positive nuclei were increased almost 3.5-fold (*P < 0.01 vs. control). Pretreatment with 100 nM spironolactone (Spiro) almost completely abolished the aldosterone-stimulated TUNEL-positive nuclei (†P < 0.01 vs. aldosterone). RU-486 had no effect on aldosterone-induced TUNEL-positive nuclei. B: representative images of TUNEL-positive nuclei induced by aldosterone. Arrows indicate TUNEL-positive nuclei present in cardiomyocytes. C: representative TUNEL and DAPI-stained images from ARVM treated with aldosterone and/or pretreatment with spironolactone or RU-486; n = 4 experiments.
surgery as previously described (19). At the end of 4 wk, mice were killed, hearts were dissected, and the left ventricle (LV) was snap-frozen in liquid nitrogen. Blood pressure, morphology, and echocardiographic measurements were performed as previously described (19).

Model B: ascending aortic constriction. AAC was performed as previously described (10). After mice were anesthetized and ventilated, AAC was performed by ascending aorta ligation around a 27-gauge needle using 7-0 silk suture. Sham-operated mice underwent a similar procedure without ligation of the ascending aorta. One week after AAC, mice were randomly assigned to regular chow or chow containing eplerenone (200 mg·kg body wt·day·1) (17). After 7 wk of eplerenone, mice were killed, hearts were dissected and the LV was snap-frozen in liquid nitrogen. Blood pressure, morphology, and

Fig. 2. A: cell viability, measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, was decreased with aldosterone treatment (1 μM) for 24 h (*P < 0.01 vs. control). Pretreatment with SP-600125 (SP; 2 μM), a c-Jun NH2-terminal kinase (JNK) inhibitor, increased % of viable cells (**)P < 0.05 vs. aldosterone). However, pretreatment with SB-203580 (SB; 3 μM), a p38 inhibitor, did not increase cell viability; n = 3 experiments. B: p38 and JNK signaling in aldosterone-induced apoptosis. TUNEL-positive nuclei were increased after 24 h treatment with aldosterone (*P < 0.01 vs. control). Pretreatment with SP-600125 significantly decreased % of aldosterone-induced TUNEL-positive nuclei from 22 ± 2% to 9 ± 1% in ARVM (†P < 0.01 vs. aldosterone). However, pretreatment with SB-203580 did not reduce % of aldosterone-induced TUNEL-positive nuclei in ARVM; n = 4 or 5 experiments. C: cell death as measured by nucleosome fragmentation was increased in aldosterone (P < 0.01 vs. control) and significantly decreased by pretreatment with SP-600125 (**)P < 0.05 vs. aldosterone), n = 3 experiments. D: effects of actinomycin D and cycloheximide on aldosterone-induced apoptosis. Actinomycin D (ActinD, 5 μg/ml) and cycloheximide (Cyclo, 10 μg/ml), inhibitors of RNA and protein synthesis, inhibited aldosterone-induced TUNEL-positive nuclei. (*P < 0.01 vs. control; **)P < 0.05 vs. aldosterone); n = 5 experiments.
Echocardiographic measurements were performed as previously described (17).

Statistical Analysis

All data are presented as means ± SE; differences among multiple conditions were determined by ANOVA followed by a paired t-test with the Bonferroni correction for multiple comparisons. P values <0.05 were considered significant.

RESULTS

Aldosterone Induces TUNEL-Positive Nuclei in ARVM Via MR

Aldosterone (1 μM) treatment of ARVM for 24 h significantly increased the percentage of TUNEL-positive nuclei from 6 ± 1% to 22 ± 2%. Spironolactone (100 nM) added for 30 min before aldosterone stimulation significantly decreased the percentage of TUNEL-positive nuclei to 9 ± 1%. Similarly, pretreatment with RU-486 (1 μM), a glucocorticoid receptor (GR) antagonist did not inhibit TUNEL-positive nuclei (Fig. 1).

Aldosterone Decreased Cell Viability and Induced Apoptosis Via JNK Phosphorylation

Aldosterone treatment (1 μM) for 24 h decreased total cell viability but was ameliorated by the pretreatment with SP-600125 (2 μM), a JNK inhibitor, and not with SB-203580 (3 μM), a p38 inhibitor (Fig. 2A). To determine whether the decrease in cell viability was due to cardiomyocyte apoptosis, SB-203580, the p38 inhibitor was used and had minimal effect on aldosterone-induced TUNEL-positive nuclei. However, pretreatment of ARVM with SP-600125 before aldosterone stimulation significantly decreased the percentage of TUNEL-positive nuclei (Fig. 2B). To corroborate the effects of SP-600125 on aldosterone-induced apoptosis, nucleosome fragmentation was measured by enzyme-linked immunosorbent assay. Aldosterone-induced nucleosome fragmentation was significantly suppressed with SP-600125 (Fig. 2C).

Actinomycin D and cycloheximide, inhibitors of transcription and translation, inhibited aldosterone-induced TUNEL-positive nuclei, indicating that the effect is genomically mediated. (Fig. 2D).

Aldosterone-Stimulated TUNEL-Positive Nuclei Are Inhibited by PPARα Agonist Fenofibrate

Aldosterone treatment (1 μM) for 24 h decreased total cell viability but was ameliorated by pretreatment with fenofibrate (10 μM) for 30 min (Fig. 3, A–C). Pretreatment with fenofibrate (10 μM) for 30 min before aldosterone stimulation significantly decreased the percentage of TUNEL-positive nuclei from 20 ± 1% to 15 ± 1% in ARVM. Pretreatment with 100 μM fenofibrate for 30 min before 24 h of aldosterone incubation resulted in complete necrosis of ARVM (data not shown). Additionally, aldosterone also increased p53 expression in ARVM, while pretreatment with fenofibrate decreased the aldosterone-induced increase in p53 expression in ARVM (Fig. 3, D and E).

Stress-Activated Kinases Mediate Fenofibrate’s Inhibition of Aldosterone-Induced Apoptosis in ARVM

By Western blotting, aldosterone (1 μM) significantly induced JNK phosphorylation in ARVM. This expression was inhibited 33 ± 6% by pretreatment with fenofibrate (Fig. 4, A and B). Aldosterone significantly induced p38 phosphorylation in ARVM, which was also significantly decreased with fenofibrate (Fig. 4, C and D).

Involvement of Intrinsic (Mitochondrially Mediated) Pathway

Aldosterone-stimulated programmed cell death was associated with an increase in expression of Bax protein, a proapoptotic marker, by approximately threefold. Fenofibrate significantly inhibited Bax expression in aldosterone-stimulated ARVM (Fig. 5A). Similarly, aldosterone-mediated apoptosis was associated with an approximately twofold decrease in Bcl-2 protein, an antiapoptotic marker. We further demonstrated that fenofibrate increased Bcl-2 expression in aldosterone-stimulated ARVM (Fig. 5B).

Cleaved caspase-3 protein expression was measured, and its cleavage into 19- and 17-kDa fragments resulted in DNA fragmentation. Aldosterone significantly increased expression of cleaved caspase-3 (the active form of caspase-3) in ARVM and was significantly decreased by pretreatment with fenofibrate (Fig. 5, C and D).

We then sought to determine the significance of these findings in two in vivo animal models: aldosterone infusion, uninephrectomy, and 1% NaCl (model A) and pressure overload (PO) induced by AAC (model B).

Chronic Aldosterone Infusion and Fenofibrate

We previously demonstrated (19) that fenofibrate attenuated adverse cardiac remodeling induced by chronic aldosterone administration. Fenofibrate reduced cardiac hypertrophy, altered MMPs and tissue inhibitors of MMP (TIMPs), and decreased myocardial fibrosis and lipid accumulation (19). We sought to determine the myocardial expression of the Bcl-2 family proteins in the LV. Four weeks after chronic aldosterone, myocardial Bax expression was increased 58 ± 2% and subsequently decreased −39 ± 2% when aldosterone-infused mice were treated with fenofibrate (Fig. 6, A and B). Interestingly, aldosterone infusion had a negligible effect on Bcl-2 expression, but in aldosterone-infused mice treated with fenofibrate Bcl-2 expression was increased.

*Fig. 3. A: fenofibrate inhibited the aldosterone-induced decrease in cell viability in ARVM. Aldosterone (1 μM) for 24 h decreased cell viability (**P < 0.01 vs. control) measured with the MTT assay. Thirty minutes with 10 μM fenofibrate (Fen-10) did not affect cell viability. However, pretreatment with fenofibrate (10 μM) for 30 min before aldosterone stimulation (Fen-10 + Aldo) significantly increased cell viability (**P < 0.05 vs. aldosterone). B: fenofibrate ameliorated the aldosterone-induced TUNEL-positive nuclei in ARVM. Pretreatment with fenofibrate (10 μM) decreased % TUNEL-positive nuclei induced by aldosterone (**P < 0.05 vs. aldosterone; P < 0.001 vs. control); n = 4 experiments. C: representative TUNEL and DAPI-stained images from ARVM treated with aldosterone and/or pretreated with fenofibrate (10 μM). D: fenofibrate decreased aldosterone-induced p53 expression in ARVM. Aldosterone (1 μM) for 30 min increased p53 expression by 58 ± 8% (§P < 0.001 vs. control). However, pretreatment with fenofibrate (10 μM) for 30 min significantly decreased this increase in p53 expression in ARVM by 42 ± 17% (***P < 0.05 vs. aldosterone). E: representative immunoblot of p53 expression.*
was increased by 89 ± 2% vs. untreated aldosterone mice (Fig. 6, C and D).

**Pressure Overload and Eplerenone**

Eplerenone, the selective MR antagonist, ameliorated progressive LV remodeling and systolic dysfunction in PO-induced cardiac remodeling (17). Eplerenone inhibited MMP activity and TUNEL-positive nuclei and was accompanied by a decrease in myocardial oxidative stress and inflammation in the LV (17). PO resulted in a ~2.5-fold increase in myocardial Bax expression that was significantly decreased after chronic eplerenone treatment (Fig. 7, A and B). Interestingly, there was no increase in myocardial Bcl-2 protein expression in either eplerenone-treated or untreated PO hearts (Fig. 7, C and D). However, the Bcl-2-to-Bax ratio, an indicator of the net antiapoptotic effect, was significantly increased in eplerenone-treated PO hearts vs. untreated PO hearts (Fig. 7E).
DISCUSSION

The major findings in our study were as follows: 1) In cultured ARVM, aldosterone stimulation increased TUNEL-positive nuclei that were mediated via the MR and not by the GR. 2) Our data further indicate that aldosterone-mediated apoptosis in ARVM was associated with expression of JNK signaling pathways. 3) The PPARα activator fenofibrate inhibited TUNEL-positive nuclei in ARVM by opposing the proapoptotic action of JNK expression. 4) Finally, aldosterone affected proapoptotic mitochondrial proteins by increasing Bax and decreasing Bcl-2 protein expression in ARVM. This was reversed with fenofibrate pretreatment. In concert, an in vivo model using chronic fenofibrate (19) reversed the cardiac expression of Bax and Bcl-2 proteins. Similarly eplerenone, which previously also demonstrated improved cardiac remodeling (17), also reversed cardiac Bax expression but had no effect on Bcl-2 protein and resulted in a net increase in the Bcl-2-to-Bax ratio.

Aldosterone-Induced Apoptosis

In human heart failure, plasma levels of aldosterone are ~10^{-7} mol/l (10^{-1} μM or 100 nM); however, myocardial aldosterone levels are ~17 times higher than that in plasma (24, 35, 38). Therefore, 1 μM is a clinically relevant dose. Several explanations for this graded concentration effect have
been suggested, e.g., aldosterone degradation may be slower in myocardium than in plasma or may be isolated intracellularly and/or locally delivered into the extracellular space instead of being released into plasma (38). Similar results have been reported for other neurohormones, e.g., angiotensin II (8) and angiotensin I (9).

We previously showed (37) that aldosterone infusion induced myocardial apoptosis. In the present study, aldosterone-mediated apoptosis in ARVM is mediated by JNK signaling pathways. The proposed role of JNK and p38 as positive regulators of cardiac hypertrophy in vivo is controversial (22). The prohypertrophic regulatory role of aldosterone was defined in NRVM (27, 28). In NRVM aldosterone increases JNK activation (27) or augments endothelin-1-induced JNK activation (28) and results in hypertrophy. As our data demonstrate, the stress-activated protein kinases JNK and p38 may actually serve as negative regulators of cardiac remodeling in the heart (22). JNK and p38 are described as key mediators of the stress signaling pathway in cardiomyocyte apoptosis (1).

JNK is proapoptotic in many cell types, and its activation correlates with cardiomyocyte apoptosis in response to mechanical stress, cytokines, and oxidative stress. Interestingly in NRVM, aldosterone-induced apoptosis involves calcineurin activation (24) and it occurs via a rapid nongenomic response resulting in stimulation of the mitochondrial apoptotic pathway that is associated with calcineurin signaling (24).

In our study, the p38 inhibitor SB-203580 did not inhibit cardiomyocyte apoptosis, indicating that p38 MAP kinase does not play a role in aldosterone-induced apoptosis in ARVM. Proapoptotic and antiapoptotic actions have both been ascribed to p38 and likely depend on the intensity and the duration of p38 activation and cardiomyocyte susceptibility to apoptosis. Proapoptotic activation of p38 has been shown to occur during ischemia (3) or with anthracyclines (44). Conversely, p38 activation protects against β-adrenergic-induced apoptosis (6). In our study p38 MAP kinase expression was not involved in aldosterone-induced apoptosis but may be involved in another prosurvival pathway because its expression by aldosterone is suppressed by fenofibrate.

Aldosterone in cardiomyocytes mediates effects on mitochondrial proteins by increasing Bax and decreasing Bcl-2 expression in both in vivo and in vitro models. Interestingly, in mice subjected to chronic PO, selective inhibition of the MR had no effect on Bcl-2 expression, although eplerenone previously decreased TUNEL-positive nuclei (17). Because of its effect in decreasing Bax expression, the Bcl-2-to-Bax ratio is increased with eplerenone treatment, indicating a net protective effect of selective MR inhibition. The changes in the Bcl-2 family of proteins are attributed to the increase in cardiomyocytes undergoing apoptosis (21). Aldosterone affects other antiapoptotic proteins such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), tumor necrosis factor-related weak inducer of apoptosis (TWEAK), and Fas ligand in other cell systems (25). Similar to others (29, 42), we now demonstrate that aldosterone also induces other proapoptotic proteins, e.g., cleaved caspase-3 and p53 in cardiomyocytes. Cleaved caspase-3 is downstream of the Bcl-2 family apoptotic cascade and integrates apoptotic signaling. Additionally, aldosterone induces the production of reactive oxygen spe-
cies (36), which may be directly responsible for apoptosis in many cell types (18, 29).

**Fenofibrate Inhibits Aldosterone-Induced Apoptosis**

In ARVM, fenofibrate inhibited aldosterone-induced MMP activity and ERK phosphorylation (10). Furthermore, we reported (19) that fenofibrate improved aldosterone-induced myocardial remodeling, with decreased fibrosis and modification of the extracellular matrix. We therefore investigated the effects of fenofibrate on aldosterone-induced apoptosis in vitro. In our study the number of apoptotic nuclei remained at basal levels using a low dose of fenofibrate (10 nM). Pretreatment of ARVM with this dose of fenofibrate significantly inhibited aldosterone-induced apoptosis. Necrosis was seen in ARVM with a high dose of fenofibrate (100 nM) (data not shown). These findings are in accordance with prior reports in which fenofibrate displayed either proapoptotic (16, 40) or antiapoptotic (42) effects in different cells, e.g., fenofibrate inhibited apoptosis (10).
palmitate-induced apoptosis in chick cardiomyocytes (15). At high concentrations, while fenofibrate causes cell injury, lower doses of fenofibrate may promote cell survival via AMPK-activated protein kinase (AMPK)-dependent pathway (14). Fenofibrate has been shown to increase adiponectin levels in patients with hypertriglyceridemia and the metabolic syndrome (34). Although not tested in our study, the antiapoptotic effects of fenofibrate in cardiomyocytes may be due to increased adiponectin.

We next examined the consequences of fenofibrate treatment leading to the survival of aldosterone-treated ARVM. In the present study aldosterone induced the stress-activated protein kinases p38 and JNK, which were decreased with low-dose fenofibrate in vitro. Others have shown that low-dose fenofibrate inhibited p38 and JNK expression in endothelin-1-stimulated NRVM (13). To investigate whether fenofibrate would inhibit the apoptotic signaling components downstream of the stress-activated protein kinases, we investigated whether fenofibrate could affect other regulators of stress-induced apoptosis, such as the anti- and proapoptotic Bcl-2 family members Bcl-2/Bax. Fenofibrate increased Bcl-2 and decreased Bax expression in myocardial tissue, which correlated with in vitro investigations. In contrast Kubota et al. (16) reported that fenofibrate was proapoptotic with decreased Bcl-2 gene expression in human hepatocytes. This may relate to the cell type since other fibrates have been shown to inhibit the translocation of proapoptotic Bax from the cytosol to the mitochondrial fraction and increased the expression of antiapoptotic Bcl-2 (26). Fibrates also prevent the release of cytochrome c from the mitochondria and the cleavage of procaspase-3 to active caspase-3 (26). Our study now demonstrates that fibrates also inhibit the aldosterone-induced increase in cleaved caspase-3 expression in cardiomyocytes. Additionally, fenofibrate also decreases the aldosterone-induced p53 expression in cardiomyocytes. p53, a transcription factor, mediates apoptosis in response to diverse stimuli and DNA damage and may transactivate the expression of multiple proapoptotic genes including MAPK family and Bcl-2 family proteins (7).

Although fenofibrate is both a fibrate and a PPARα activator, it is also a weak activator of PPARγ/β and in other cell types activates other pathways that are not PPAR dependent, such as non-receptor-mediated (nongenomic) effects (30) and non-PPARα-mediated effects (such as inhibiting Akt phosphorylation, resulting in apoptosis) (16). Our present findings do not differentiate between direct or indirect effects of fenofibrate.

Our data therefore suggest that aldosterone (1 μM) induced apoptosis via a stress kinase signaling pathway and the Bcl-2 family components. Fenofibrate, a PPARα agonist, inhibited aldosterone-induced apoptosis in cardiomyocytes. Our present findings help to elucidate the stress signaling pathways that exert aldosterone-induced apoptotic effects in cardiomyocytes. These findings suggest that the regulation of apoptosis by aldosterone involves differential expression of stress kinase signaling pathways. Additionally, fenofibrate exerts effects with the net result reflecting a balance between the antiapoptotic effect via JNK and the antiapoptotic actions of ERK1/2 (10).

We previously showed that fenofibrate inhibits aldosterone-stimulated ERK1/2 phosphorylation and MMPs in PO cardiac remodeling (10). Likewise in aldosterone-induced cardiac remodeling, fenofibrate decreased lipid accumulation and the expression of PPARα targets: UCP3 and MCAD (19). Our present study extends the findings of the involvement of the JNK MAPK-dependent mitochondrial death pathway in aldosterone-induced apoptosis and may be directly inhibited by fenofibrate.

Our findings indicate that aldosterone (although inducing p38 expression) mediates proapoptotic protein expression via JNK MAPK signaling pathways. Cross talk exists between fenofibrate, and possibly PPARα, and the MR pathways (20). Our findings suggest that fenofibrate is important in determining the myocyte phenotype via the differential activation and interplay of multiple kinase signaling pathways. The present findings indicate the complex role that aldosterone and PPARα agonists play in mediating an adult cardiomyocyte phenotype and provide a mechanism by which a stimulus may exert diverse effects on cardiomyocyte phenotype and provide a mechanism by which a stimulus may exert diverse effects on cardiomyocyte hypertrophy (19) and apoptosis. These findings suggest that PPARα ligands may modulate hypertrophy and myocardial remodeling specifically by antiapoptotic effects in aldosterone-mediated hypertension and cardiac remodeling.

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