

Myocardial Fibrosis and Diastolic Dysfunction in Deoxycorticosterone Acetate-Salt Hypertensive Rats Is Ameliorated by the Peroxisome Proliferator-Activated Receptor-Alpha Activator Fenofibrate, Partly by Suppressing Inflammatory Responses Associated With the Nuclear Factor-Kappa-B Pathway

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- OBJECTIVES** We sought to clarify that a peroxisome proliferator-activated receptor-alpha (PPAR-alpha) activator inhibits myocardial fibrosis and its resultant diastolic dysfunction in hypertensive heart disease, as well as to investigate whether inflammatory mediators through the nuclear factor (NF)-kappa-B pathway are involved in the effects.
- BACKGROUND** Patients with hypertensive heart disease often have diastolic heart failure without systolic dysfunction. Meanwhile, it has been well established in atherosclerosis that PPAR-alpha activation negatively regulates early inflammation. In hypertensive hearts, however, it is still unclear whether PPAR-alpha activation inhibits inflammation and fibrosis.
- METHODS** Twenty-one rats were randomly separated into the following three groups: deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with a PPAR-alpha activator, fenofibrate (80 mg/kg/day for 5 weeks); DOCA-salt rats treated with vehicle only; and uninephrectomized rats as normotensive controls.
- RESULTS** Fenofibrate significantly inhibited the elevation of left ventricular end-diastolic pressure and the reduction of the magnitude of the negative maximum rate of left ventricular pressure rise and decline, corrected by left ventricular pressure ($-dP/dt_{max}/P$), which are indicators of diastolic dysfunction. Next, fenofibrate prevented myocardial fibrosis and reduced the hydroxyproline content and procollagen I and III messenger ribonucleic acid expression. Finally, inflammatory gene expression associated with NF-kappa-B (interleukin-6, cyclooxygenase-2, vascular cell adhesion molecule-1, and monocyte chemoattractant protein-1), which is upregulated in DOCA-salt rats, was significantly suppressed by fenofibrate. Activation of NF-kappa-B and expression of I-kappa-B-alpha in DOCA-salt rats were normalized by fenofibrate.
- CONCLUSIONS** A PPAR-alpha activator reduced myocardial fibrosis and prevented the development of diastolic dysfunction in DOCA-salt rats. The effects of a PPAR-alpha activator may be mediated partly by prevention of inflammatory mediators through the NF-kappa-B pathway. These results suggest that treatment with PPAR-alpha activators will improve diastolic dysfunction in hypertensive heart disease. (J Am Coll Cardiol 2004;43:1481-8) © 2004 by the American College of Cardiology Foundation

Diastolic heart failure (HF) without left ventricular (LV) systolic dysfunction comprises 30% to 50% of HF in clinical practice, and hypertensive heart disease is a major cause of this type of HF (1). The complication of myocardial fibrosis should be avoided in hypertensive heart disease, because increasing ventricular stiffness caused by myocardial fibrosis leads to the development of diastolic dysfunction of the heart. Diastolic dysfunction in patients with prolonged hypertension is often associated with myocardial fibrosis in

addition to muscular hypertrophy as a final feature of hypertensive heart disease.

Peroxisome proliferator-activated receptors (PPARs) belong to a superfamily of nuclear ligand-activated transcription factors. The PPARs are key players in lipid and glucose metabolism and are implicated in metabolic disorders predisposing to atherosclerosis (2). At present, three major PPAR family members have been identified: PPAR-alpha is highly expressed in the liver and to a lesser extent in the heart, skeletal muscle, and kidney; PPAR-gamma is predominantly expressed in adipose tissue; and PPAR-delta is ubiquitously expressed. Whereas PPAR-gamma promotes lipid storage by regulating adipocyte differentiation, PPAR-alpha stimulates beta-oxidative degradation of fatty acids.

Mice deficient in PPAR-alpha a prolonged response to inflammatory stimuli and age-dependent myocardial fibro-

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Abbreviations and Acronyms

COX-2	= cyclooxygenase-2
DOCA	= deoxycorticosterone acetate
DOCA-F	= deoxycorticosterone acetate-salt rats treated with fenofibrate
DOCA-V	= deoxycorticosterone acetate-salt rats treated with vehicle
$\pm dP/dt_{max}$	= maximum rate of left ventricular pressure rise or decline
HF	= heart failure
IL-6	= interleukin-6
LV	= left ventricle/ventricular
LVEDP	= left ventricular end-diastolic pressure
MCP-1	= monocyte chemoattractant protein-1
NF	= nuclear factor
PPAR-alpha	= peroxisome proliferators-activated receptor-alpha
UN	= uni-nephrectomized control
VCAM-1	= vascular cell adhesion molecule-1

sis, suggesting that PPAR-alpha is also a modulator of inflammation and fibrosis (3,4). Hypolipidemic fibrate drugs are PPAR-alpha ligands that inhibit the progressive formation of atherosclerotic lesions, which involves chronic inflammatory processes (5), even in the absence of an atherogenic lipoprotein-lowering effect (6,7). Various data suggest that fibrates inhibit the vascular inflammatory response via PPAR-alpha by interfering with the nuclear factor (NF)-kappa-B transactivation capacity, involving a direct protein-protein interaction with the p65 subunit (8,9). Our preliminary study demonstrated that a PPAR-alpha activator prevented myocardial fibrosis in the heart of hypertrophied rats after abdominal aortic banding (10), but we did not clarify the mechanism by which a PPAR-alpha activator prevented myocardial fibrosis and whether it improved diastolic dysfunction of the heart in that model.

Therefore, the purpose of this study was to examine the effects of a PPAR-alpha activator, fenofibrate, on myocardial fibrosis and diastolic dysfunction in a deoxycorticosterone acetate (DOCA)-salt hypertensive rat model. The reason we used DOCA-salt rats for the present study is that rats with prolonged hypertension provided a more suitable model of human hypertensive heart disease. Furthermore, it is important to investigate the effects of fenofibrate with respect to the inflammatory response contributing to myocardial fibrosis, because Ammarguella et al. (11) suggested that activation of inflammatory mediators related to NF-kappa-B could be major components of myocardial fibrosis in this model. Therefore, we examined the gene expression of inflammatory cytokines that are regulated by NF-kappa-B, such as interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), vascular cell adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1), at the messenger ribonucleic acid (mRNA) level in this model, as well as the effects of a PPAR-alpha activator on both NF-kappa-B activation and I-kappa-B-alpha protein expression.

METHODS

Animal models and experimental designs. Twenty-one male Sprague-Dawley rats (weighing 160 to 180 g, age 6 weeks) were randomly separated into three groups: the first group comprised DOCA-salt rats treated with fenofibrate (DOCA-F) (n = 8; 80 mg/kg/day by gavage; Kaken Seiyaku Ltd., Tokyo, Japan) in vehicle (3% arabic gum); the second group comprised DOCA-salt rats treated with vehicle alone (DOCA-V) (n = 7); and the third group comprised only uni-nephrectomized rats (UN control, n = 6). The protocol for the DOCA-salt rats was carried out according to the method of Matsumura et al. (12); after one-week recovery following unilateral nephrectomy, rats were subcutaneously injected with DOCA (15 mg/kg; Sigma, Tokyo, Japan) suspended in corn oil twice a week and given 1% NaCl drinking water for five weeks. The dose of fenofibrate used in the present study caused sustained activation of PPAR-alpha, without significant lipoprotein effects in normolipidemic rats (13). The rats' systolic blood pressure was monitored once a week by a tail-cuff method. This study was approved by the University of Tsukuba and conformed to the "Position of the American Heart Association on Research Animal Use," adopted by the Association in November 1984.

Hemodynamic studies and tissue sampling. All rats were sacrificed five weeks after surgery. A 2.0F, high-fidelity, manometer-tipped catheter (SPR-402, Millar Instruments, Houston, Texas) was introduced through the right carotid artery into the LV under pentobarbital anesthesia (50 mg/kg, intraperitoneally). Tracings of LV pressure were digitized at a rate of 1,000 samples/s with a commercial analog-to-digital converter (MP100WS, BIOPAC Systems Inc., Goleta, California) and a personal computer using dedicated software (Acknowledge III, Version 3, BIOPAC Systems Inc., Goleta, California). Left ventricular end-diastolic pressure (LVEDP) was determined as the pressure just before the onset of an increase in LV systolic pressure (14,15). After measurement of LV pressure, the rats were sacrificed. According to quote papers (16-18), the heart was excised and the ventricles were divided into the LV, including the interventricular septum, and the right ventricle. The ventricular samples were rinsed with cold saline, weighed, quickly frozen in liquid nitrogen, and stored at -80°C. Left ventricular hypertrophy was evaluated by the LV wet weight to body weight ratio.

Histological studies. For histologic analysis, the hearts were subsequently fixed in 4% paraformaldehyde and embedded in paraffin. Coded slices stained with Masson trichrome were microscopically evaluated for interstitial and perivascular myocardial collagen deposition.

Hydroxyproline content in myocardial extracts. The hydroxyproline content in myocardial extracts was measured and used as an index of collagen content. The apical site of the LV below the papillary muscles was removed, immediately placed in liquid nitrogen, and stored at -80°C until measurement of the hydroxyproline content, according to

the method of Stegemann and Stalder (19). The results were calculated as hydroxyproline content per wet weight of tissue.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of procollagen I and III, IL-6, COX-2, VCAM-1, and MCP-1 mRNA. To evaluate whether fenofibrate ameliorates myocardial fibrosis not only at the histologic and hydroxyproline levels but also at the molecular level, we investigated mRNA expression of procollagen I and III in the LV of the three groups. Furthermore, mRNA expression of IL-6, COX-2, VCAM-1, and MCP-1 was determined. The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also determined as an internal control. Total RNA from the LV was isolated by the method of acid guanidinium thiocyanate/phenol/chloroform extraction with ISOGEN (Nippon Gene Ltd., Tokyo, Japan), according to methods described in our previous reports (16–18). Semiquantitative RT-PCR was also performed according to our previous article (20). The sequences of the specific primers were as follows: procollagen I (sense): 5'CATAAAGGGT-CATCGTGGCTTC3'; procollagen I (antisense): 5'GT-GATAGGTGATGTTCTGGAG3'; procollagen III (sense): 5'GTGACAGAGGTGAAAGAGGATC3'; procollagen III (antisense): 5'CTGTCTTGCTCCATTCAC-CAGT3'; IL-6 (sense): 5'GTATGAACAGCGAT-GATGC3'; IL-6 (antisense): 5'CATATTGCCAGT-TCTTCGTA3'; COX-2 (sense): 5'GTATGACACAA-CAGCCATCTC3'; COX-2 (antisense): 5'CTTGTC-AACCCCTCCAATT3'; VCAM-1 (sense): 5'CCT-GAACTCCTTGACTCTACT3'; VCAM-1 (antisense): 5'CAACAGTCAGTCCAAGCAACAC3'; MCP-1 (sense): 5'ACCTGCTGCTACTCATTCACTG3'; MCP-1 (antisense): 5'GTGGTTGTGGAAAAGAGA-GTGG3'; GAPDH (sense): 5'GCCATCAACGAC-CCCTTCATTG3'; GAPDH (antisense): 5'TGC-CAGTGAGCTTCCCCTTC3'.

Polymerase chain reaction was performed with the annealing temperature and required cycles for each template as follows: 59°C for procollagen I, 62°C for procollagen III, 62°C for IL-6, 59°C for COX-2, 61°C for VCAM-1, 62°C for MCP-1, and 62°C for GAPDH. The amplified products on agarose gel were stained with ethidium bromide, visualized with a ultraviolet transilluminator, and photographed. The photographs were scanned (CanoScan 600, Canon Ltd., Tokyo, Japan), and quantification was performed with MacBAS (Fuji Film Ltd., Tokyo, Japan).

Detection of NF-kappa-B activation. To determine NF-kappa-B activity, we used a BD Mercury TransFactor kit (BD Biosciences Clontech, Tokyo, Japan). Using an ELISA-based format, the TransFactor kit detected DNA binding by specific transcription factors (21). The consensus binding sequence for the NF-kappa-B p65 subunit was GGGGTATTTCC (22). Absorbance was measured at 655 nm with a microplate reader (Biolumin 960, Molecular Dynamics, Inc., Sunnyvale, California). Five rats in each group were used, and 100 µg nuclear

protein was applied for each sample. Nuclear proteins were isolated from the heart to estimate NF-kappa-B translocation. In short, 70 mg of LV from heart tissue was homogenized in ice-cold Tris-buffered saline and centrifuged at 3,000g for 5 min at 4°C. The pellet was resuspended by gentle pipetting in 0.8 ml of ice-cold hypotonic buffer containing 10 mmol/l HEPES, pH 7.9, 10 mmol/l KCl, 0.1 mmol/l EDTA, 0.1 mmol/l EGTA, 1 mmol/l dithiothreitol, 0.5 mmol/l phenylmethane sulfonyl fluoride, 50 mmol/l NaF, 30 mmol/l beta-glycerolphosphate, 1 mmol/l Na₃VO₄, and 10 µg/ml each of aprotinin, pepstatin, and leupeptin. The solution was allowed to swell on ice for 15 min after addition of 100 µl of 10% Nonidet P-40 (Wako, Osaka, Japan), and the tube was vortexed vigorously for 45 s. The homogenate was centrifuged for 30 s at 4°C in a microcentrifuge tube. The supernatant containing cytoplasmic protein was stored at -80°C. The nuclear pellet was resuspended in a solution containing 20 mmol/l HEPES, pH 7.9, 0.4 mol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l DTT, 1 mmol/l PMSF, and 10 µg/ml each of aprotinin, pepstatin, and leupeptin. The tubes were shaken vigorously at 4°C for 30 min on a shaking platform. The nuclear extracts were stored at -70°C. The protein concentration was estimated using a Pierce protein assay kit (Wako, Osaka, Japan).

Western blot analysis of I-kappa-B-alpha. Cytoplasmic proteins (10 µg) from each sample were mixed with sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) sample buffer containing 62 mmol/l Tris, pH 6.8, 10% glycerol, 2% SDS, 5% beta-mercaptoethanol, and 0.003% bromophenol blue, heated at 95°C for 5 min and separated by 12.5% SDS-PAGE electrophoresis. The proteins were transferred onto Immobilon transfer membranes (Millipore, Tokyo, Japan). The I-kappa-B proteins were detected using I-kappa-B-alpha antibody (New England Biolabs, Tokyo, Japan), horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Amersham, Tokyo, Japan) and Enhanced ChemiLuminescence (ECL plus from Amersham) reagents.

Statistical analysis. All data are expressed as the mean value ± SEM. One-way analysis of variance followed by Bonferroni correction for post hoc tests was used for statistical comparison among the various treatment groups. All statistical comparisons were performed with StatView version 5.0 for Windows (SAS Institute Inc., Cary, North Carolina). Values of *p* < 0.05 were considered statistically significant.

RESULTS

Systolic blood pressure, body weight, and heart weight of rats. Time-related changes in systolic blood pressure for the three groups are shown in Figure 1. Systolic blood pressure in DOCA-V and DOCA-F rats was elevated to a similar degree after two weeks and was significantly higher than that in UN control rats after four weeks (184 ± 10 and 178 ± 6 vs. 144 ± 5 mm Hg, respectively; *p* < 0.01). Body

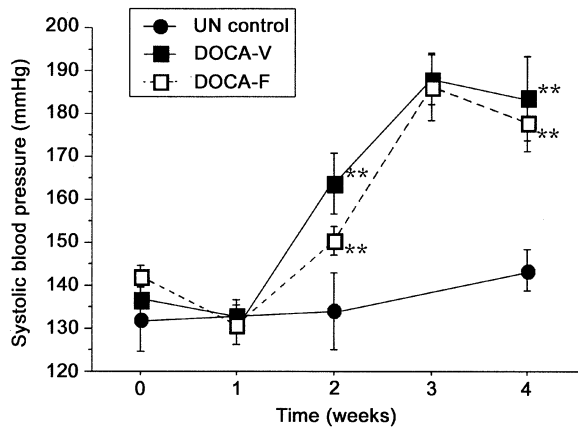


Figure 1. Time-related changes in systolic blood pressure of uninephrectomized control rats (UN control) (n = 6), deoxycorticosterone acetate-salt rats treated with vehicle (DOCA-V) (n = 7), and deoxycorticosterone acetate-salt rats treated with fenofibrate (DOCA-F) (n = 8) rats. Data are expressed as the mean value ± SEM. **p < 0.01 vs. UN control.

weight was similar in UN control and DOCA-salt rats and was unaffected by administration of fenofibrate. Body weight-corrected LV mass was significantly increased in DOCA-V and DOCA-F rats compared with UN control rats, and there was no difference in LV mass between DOCA-F and DOCA-V rats (Table 1).

Effects of fenofibrate on hemodynamics in DOCA-salt rats. We measured the negative or positive maximum rate of LV pressure rise or decline ($\pm dP/dt_{max}$), the value corrected by the corresponding LV pressure ($\pm dP/dt_{max}/P$), and LVEDP in the three groups (Table 2). The DOCA-V rats had a significant decrease in $-dP/dt_{max}/P$ and a significant increase in LVEDP, as compared with UN control rats (p < 0.01). These parameters were improved by fenofibrate treatment (p < 0.01 vs. DOCA-V). The values of $+dP/dt_{max}/P$ were similar among the three groups.

Effects of fenofibrate on collagen deposition and procollagen I and III mRNA expression in the LV of DOCA-salt rats. Figure 2 shows representative photomicrographs of the myocardium of rats in the UN control, DOCA-V, and DOCA-F groups. There appears to be an increase in interstitial and perivascular collagen deposi-

Table 1. Body and Heart Weights of Deoxycorticosterone Acetate-Salt Rats Treated With the Peroxisome Proliferator-Activated Receptor-Alpha Activator, Fenofibrate

	UN control (n = 6)	DOCA-V (n = 7)	DOCA-F (n = 8)
BW (g)	414 ± 8	388 ± 24	409 ± 14
LVw (mg)	845 ± 23	1,100 ± 51*	1,070 ± 53*
RVw (mg)	230 ± 7	217 ± 11	245 ± 15
LVw/BW (mg/g)	2.04 ± 0.04	2.88 ± 0.16*	2.63 ± 0.13*
RVw/BW (mg/g)	0.56 ± 0.01	0.56 ± 0.02	0.60 ± 0.03

*p < 0.01 vs. UN control. Data are presented as the mean value ± SEM. BW = body weight; DOCA-F = DOCA-salt hypertensive rats treated with fenofibrate; DOCA-V = deoxycorticosterone acetate-salt hypertensive rats treated with vehicle; LVw = left ventricular wet weight; RVw = right ventricular wet weight; UN control = uni-nephrectomized control rats.

Table 2. Hemodynamics in Deoxycorticosterone Acetate-Salt Rats Treated With the Peroxisome Proliferator-Activated Receptor-Alpha Activator, Fenofibrate

	UN Control	DOCA-V	DOCA-F
Systolic BP (mm Hg)	135 ± 9	178 ± 10*	161 ± 13
+dP/dt _{max} (mm Hg/s)	4,298 ± 428	4,848 ± 336	4,251 ± 217
-dP/dt _{max} (mm Hg/s)	-3,497 ± 322	-4,085 ± 327	-3,178 ± 521
+dP/dt _{max} /P (1/s)	58.0 ± 3.4	54.4 ± 1.8	53.1 ± 1.0
-dP/dt _{max} /P (1/s)	-39.3 ± 1.2	-32.1 ± 0.9†	-37.5 ± 0.8‡
LVEDP (mm Hg)	3.4 ± 0.4	6.9 ± 0.5†	4.7 ± 0.3*‡

*p < 0.05 vs. UN control, †p < 0.01 vs. UN control, ‡p < 0.01 vs. DOCA-V. Data are presented as the mean value ± SEM.

BP = blood pressure; $\pm dP/dt_{max}$ = positive or negative maximum rate of rise and fall of left ventricular pressure; $\pm dP/dt_{max}/P$ = $\pm dP/dt_{max}$ corrected by corresponding left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; other abbreviations as in Table 1.

tion in DOCA-V compared with UN control rats. Treatment with fenofibrate clearly prevented collagen deposition in DOCA-salt rats. These findings were confirmed by analyzing the hydroxyproline content. Figure 3 shows the hydroxyproline content and levels of procollagen I and III mRNA in the LV of each group, both of which were 1.6- to 1.9-fold higher in DOCA-V than in UN control rats (p < 0.05). In DOCA-F rats, the hydroxyproline content and levels of procollagen I and III mRNA were significantly reduced compared with those in DOCA-V rats (4.68 ± 0.21 vs. 3.62 ± 0.40 μmol/g, 1.86 ± 0.22 vs. 1.11 ± 0.35 U, 1.61 ± 0.12 vs. 1.13 ± 0.12 U, respectively; p < 0.05 vs. DOCA-V).

Effects of fenofibrate on IL-6, COX-2, VCAM-1, and MCP-1 mRNA expression in the LV of DOCA-salt rats. Figure 4 shows levels of IL-6, COX-2, VCAM-1, and MCP-1 mRNA in each group. The inflammatory gene expression levels in the LV were significantly higher in DOCA-V than in UN control rats (p < 0.05). In DOCA-F rats, levels of IL-6, COX-2, VCAM-1, and MCP-1 mRNA were significantly lower than in DOCA-V rats (7.50 ± 1.34 vs. 4.01 ± 0.78, 2.62 ± 0.25 vs. 1.20 ± 0.87, 1.89 ± 0.16 vs. 1.11 ± 0.22, and 5.17 ± 1.02 vs. 2.55 ± 0.51 U, respectively; p < 0.05 vs. DOCA-V).

The DNA binding activity of NF-kappa-B and I-kappa-B-alpha protein levels in the LV of DOCA-salt rats. Because inflammatory gene expression interferes with NF-kappa-B activation, and a PPAR-alpha activator upregulates NF-kappa-B activity, we measured the DNA binding activity of NF-kappa-B using the NF-kappa-B consensus binding sequence (Fig. 5A). Activation of NF-kappa-B in DOCA-V rats was significantly higher than that in UN control rats (p < 0.05). In DOCA-salt rats treated with fenofibrate, NF-kappa-B activation returned to the basal level of UN control rats (0.118 ± 0.003 vs. 0.100 ± 0.005 optical density; p < 0.05 vs. DOCA-V). Figure 5B shows a representative example of I-kappa-B-alpha protein in each group assessed by Western blotting. Expression of I-kappa-B-alpha was clearly suppressed in DOCA-V compared with UN control rats. The protein expression in

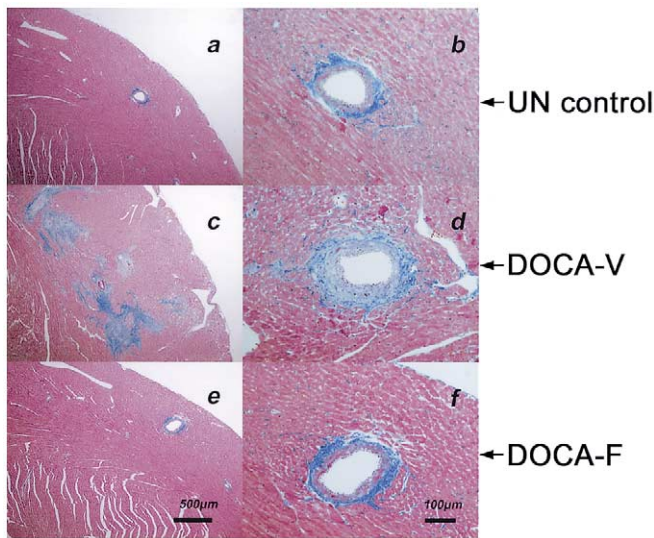


Figure 2. Interstitial and perivascular collagen deposition in the myocardial region of the left ventricle, as demonstrated by Masson trichrome staining. (a, b) UN control; (c, d) DOCA-V; and (e, f) DOCA-F. Bar indicates 500 μm (left) or 100 μm (right). Abbreviations as in Figure 1.

DOCA-F rats returned to the basal level. Similar results of Figure 5B were obtained from three independent experiments.

DISCUSSION

The present study demonstrated that long-term treatment with a PPAR-alpha activator, fenofibrate, prevented the elevation of LVEDP and the development of diastolic HF

in DOCA-salt hypertensive rats. In this model, no preventive effects on heart weight and LV systolic function were provided by fenofibrate. However, perivascular and interstitial collagen deposition was clearly reduced. Procollagen I and III mRNA levels, which were increased in the heart, were also reduced by fenofibrate. Furthermore, mRNA expression of IL-6, COX-2, VCAM-1, and MCP-1, which is upregulated in DOCA-salt rats, was significantly suppressed by fenofibrate. Activation of NF-kappa-B and the protein concentration of I-kappa-B-alpha in DOCA-salt rats were also normalized by fenofibrate. The present study suggests that activation of inflammatory mediators via the NF-kappa-B pathway is an important cause of myocardial fibrosis in this model.

Relationship of myocardial fibrosis and diastolic dysfunction. Burlaw and Weber (23) pointed out the importance of myocardial fibrosis as a cause of diastolic dysfunction. Myocardial fibrosis has been demonstrated in the postmortem human heart and in endomyocardial biopsy tissue in hypertensive heart disease. The amount of collagen present in the myocardium has also been reported to represent the most significant factor related to echocardiographic demonstration of diastolic dysfunction in hypertensive heart disease (24). In an in vivo study, Brilla et al. (25) also demonstrated a close relationship between myocardial fibrosis and myocardial diastolic dysfunction in genetic hypertensive rats with HF. The present study demonstrated both myocardial fibrosis and myocardial diastolic dysfunction in the heart of DOCA-salt hypertensive rats. These

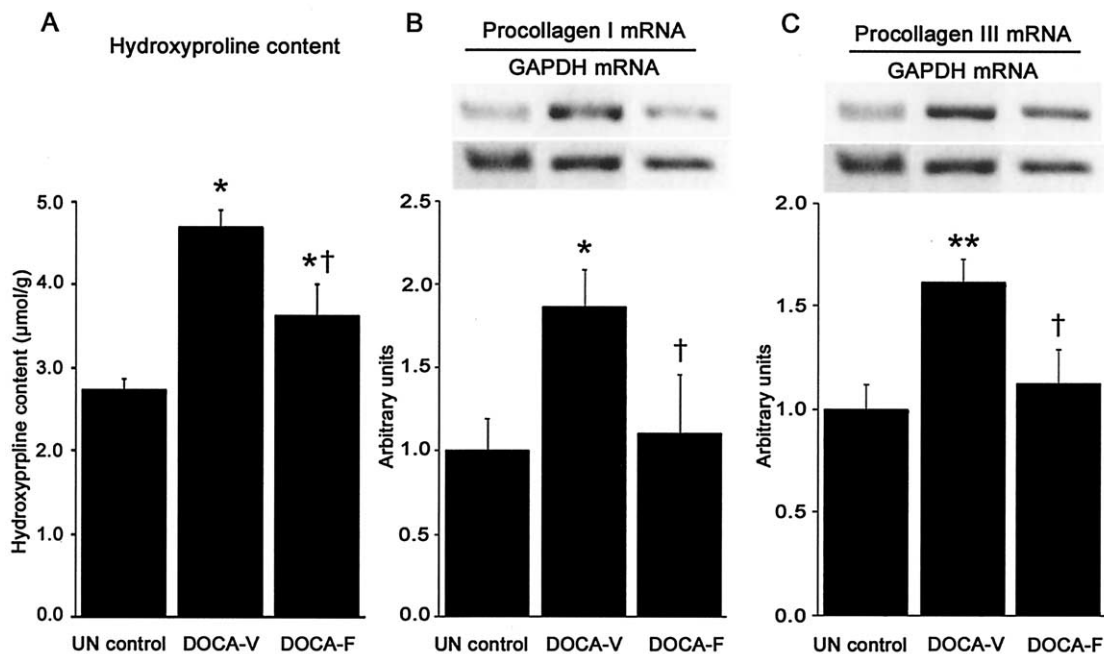


Figure 3. Effects of peroxisome proliferators-activated receptor-alpha activator on the hydroxyproline content and procollagen messenger ribonucleic acid (mRNA) expression in DOCA-salt hypertensive rats. The hydroxyproline content (A) and expression of procollagen I (B) and III (C) mRNA levels are compared among the three groups: UN control (n = 6), DOCA-V (n = 7), and DOCA-F (n = 8). Expression of mRNA was determined by reverse-transcription polymerase chain reaction, and procollagen mRNA expression was corrected by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The DOCA-F results are compared with UN control or DOCA-V and shown as the mean value \pm SEM. *p < 0.05 vs. UN control, **p < 0.01 vs. UN control, †p < 0.05 vs. DOCA-V. Abbreviations as in Figure 1.

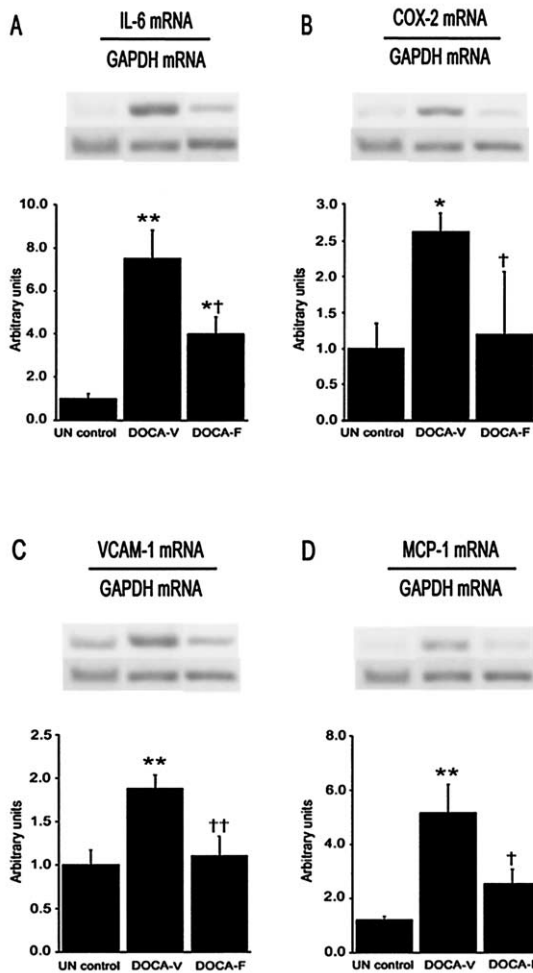


Figure 4. Effects of peroxisome proliferators-activated receptor-alpha activator on messenger ribonucleic acid (mRNA) expression of interleukin (IL)-6 (A), cyclooxygenase (COX)-2 (B), vascular cell adhesion molecule (VCAM)-1 (C), and monocyte chemoattractant protein (MCP)-1 (D) in the left ventricle of deoxycorticosterone acetate-salt hypertensive rats. For each, the mRNA levels are compared among three groups: UN control (n = 6), DOCA-V (n = 7), and DOCA-F (n = 8). Each column and bar represent the mean value ± SEM. *p < 0.05 vs. UN control, **p < 0.01 vs. UN control, †p < 0.05 vs. DOCA-V, ††p < 0.01 vs. DOCA-V. Abbreviations as in Figure 1.

results emphasize the crucial role of myocardial fibrosis in the development of diastolic HF.

In the present study, fenofibrate administration inhibited the elevation of LVEDP in DOCA-salt hypertensive rats. Iglarz et al. (26) recently reported in their study that DOCA-salt and fenofibrate did not affect LVEDP in rats. Their observations, however, were made after DOCA-salt treatment for only three weeks. The duration of their study is considered to be too short to elevate LVEDP. We performed our study after five weeks.

Myocardial fibrosis is not constantly associated with LV hypertrophy. In the present study, LV hypertrophy was found in both groups of DOCA-V and DOCA-F rats, but myocardial fibrosis was dominant only in DOCA-V rats. The present data suggest that myocardial hypertrophy itself does not necessarily induce deposition of collagen in the

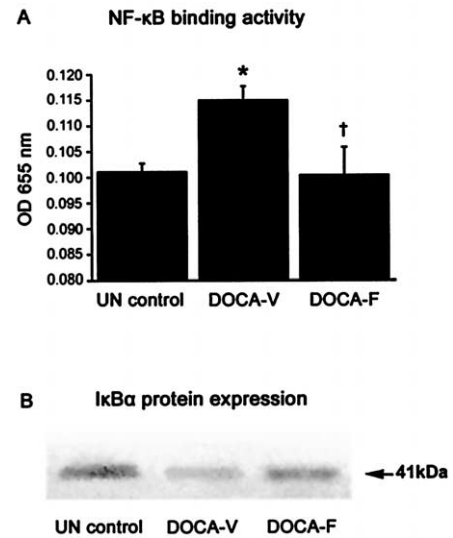


Figure 5. Effects of peroxisome proliferators-activated receptor-alpha activator on nuclear factor (NF)-kappa-B activation and I-kappa-B-alpha protein expression in deoxycorticosterone acetate-salt hypertensive rats. (A) The NF-kappa-B binding activity in the left ventricle of deoxycorticosterone acetate-salt rats. Binding specificity was assessed using an ELISA-based format, as described in the Methods. Absorbance was measured at 655 nm. Data are expressed as the mean value ± SEM. *p < 0.05 vs. UN control, †p < 0.05 vs. DOCA-V. (B) Representative Western blots of I-kappa-B-alpha protein in the left ventricle of UN control, DOCA-V, and DOCA-F rats. Abbreviations as in Figure 1.

myocardium. Nicoletti and Michel (27), in their review, argued that cardiomyocyte hypertrophy and the development of fibrosis in hypertensive heart disease are independent phenomena.

Relationship between myocardial fibrosis and inflammation. In several fibrotic processes, the role of inflammation has been clearly demonstrated. Several hypertension models revealed that perivascular fibrosis was often associated with inflammatory cell infiltration around small arteries in the myocardium (27). Profibrogenic cytokines are indeed released by inflammatory cells (28). Increased wall tension distributed throughout the whole coronary arterial tree may be involved in the extravasation of inflammatory cells around vessels, and then various cytokines from infiltrating cells, such as macrophages, become a trigger for perivascular and interstitial fibrosis. Morphologically, Hinglais et al. (29) demonstrated in the myocardium of hypertensive rats that interstitial fibroblasts expressing procollagen mRNA were localized near macrophages that infiltrated the myocardium. Since Shahar et al. (30) demonstrated that fibroblast proliferation in human interstitial lung disease was related to IL-6, inflammatory cells, such as macrophages and lymphocytes, may release cytokines that can act on cardiac resident interstitial fibroblasts.

The PPAR-alpha activator attenuates myocardial fibrosis by preventing inflammation. It has been generally accepted that NF-kappa-B is an early transcriptional factor that modulates gene expression in various situations requiring a rapid inflammatory response (31). For example, NF-kappa-B activates numerous genes, including those for adhesion molecules

involved in recruitment of circulating leukocytes to sites of inflammation. The prototypic inducible form of NF-kappa-B is a heterodimer composed of p50 (NF-kappa-B1) and p65 (Rel A) subunits, which both belong to the NF-kappa-B/Rel family of proteins. Inactive NF-kappa-B is present in the cytoplasm complexed with the inhibitory protein I-kappa-B-alpha. Nuclear factor-kappa-B is activated by a number of incoming signals from the cell surface. When released from I-kappa-B-alpha by phosphorylation via activation of I-kappa-B kinase, NF-kappa-B translocates to the nucleus and binds to the kappa-B motif of the target gene, which in turn causes activation of several factors involved in inflammatory responses.

Delerive et al. (32) demonstrated that PPAR-alpha activators induced the expression of I-kappa-B-alpha in human aortic smooth muscle cells, and they argued that the increase of I-kappa-B-alpha inhibits NF-kappa-B translocation to the nucleus and binding to the kappa-B motif. Staels et al. (2) also reported in human aortic smooth muscle cells that PPAR-alpha activation inhibits cytokine-induced activation of a number of inflammatory genes, such as VCAM-1, COX-2, and IL-6, by negatively interfering with NF-kappa-B transcriptional activity (33). Furthermore, Watanabe et al. (4) recently reported the development of myocardial fibrosis in PPAR-alpha-null mice. Therefore, we hypothesized that PPAR-alpha activators may prevent inflammation through the NF-kappa-B pathway and myocardial stiffness caused by fibrosis. In the present study, we tried to confirm this speculation in the pressure-overloaded heart of DOCA-salt rats. Activation of NF-kappa-B was increased in the heart of DOCA-salt rats, and there was upregulated mRNA expression of COX-2, VCAM-1, MCP-1, and IL-6. Furthermore, a PPAR-alpha activator, fenofibrate, attenuated NF-kappa-B nuclear binding activity and normalized cytoplasmic I-kappa-B-alpha protein expression.

Study limitations. As a limitation of this study, it should be pointed out that another mechanism exists in myocardial fibrosis besides inflammatory responses through the NF-kappa-B signaling pathway. We previously reported that fenofibrate treatment inhibited endothelin-1 mRNA levels in a model of aortic banding in rats (10). This result suggests that the PPAR-alpha activator may inhibit myocardial fibrosis by suppressing endothelin-1 production. However, the NF-kappa-B signaling pathway may have an independent association with the effect of endothelin-1, because endothelin-1 does not stimulate the NF-kappa-B pathway directly.

Conclusions. We demonstrated that prolonged hypertension induced myocardial fibrosis and diastolic dysfunction (diastolic HF), and that these disorders were clearly prevented by treatment with fenofibrate. From molecular analyses, we concluded that the effects of fenofibrate were dependent on inhibition of the inflammatory response through the NF-kappa-B pathway. Therapy with PPAR-alpha activators in patients with hypertensive heart disease may provide a new therapeutic strategy against the final development of diastolic HF in this disorder.

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