Original Article

Pioglitazone, a Thiazolidinedione Derivative, Attenuates Left Ventricular Hypertrophy and Fibrosis in Salt-Sensitive Hypertension

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Thiazolidinediones, which stimulate peroxisome proliferator–activated receptor γ, have been shown to prevent cardiovascular injury. However, little is known about their effects on salt-sensitive hypertension. We thus investigated whether or not pioglitazone affects left ventricular (LV) hypertrophy in Dahl salt-sensitive rats, then compared its effects to those of an angiotensin II receptor blocker, candesartan. Rats were used at 16 weeks of age after they had been fed either a low-salt (0.3%; DSL) or high-salt (8%; DSH) diet for 10 weeks; some of the DSH rats were treated with pioglitazone (10 mg/kg/day) or candesartan (4 mg/kg/day). Both drugs decreased the elevated blood pressure in DSH rats, although it was still higher than in DSL rats. Both drugs decreased plasma insulin levels, but neither affected plasma glucose levels. The thiobarbituric acid reactive substance level in the LV was decreased by both drugs. LV hypertrophy evaluated by echocardiography in DSH rats was nearly normalized by both drugs, whereas only candesartan decreased LV diameter. In histological analysis, both drugs ameliorated LV fibrosis and myocardial cell hypertrophy. Both drugs decreased elevated gene expression levels of transforming growth factor-β1 and collagen type I, although the pioglitazone action was slightly modest. The metalloproteinase activity was increased in DSH rats, but both drugs decreased this level. Taken together, these findings indicate that pioglitazone reduced LV hypertrophy and fibrosis in salt-sensitive hypertension. Improvement in blood pressure, insulin level, and oxidative stress may be associated with this beneficial action of pioglitazone. (*Hypertens Res* 2008; 31: 353–361)

***Key Words*: salt-sensitive hypertension, peroxisome proliferator–activated receptor γ, cardiac fibrosis, renin-angiotensin system**

Introduction

Left ventricular (LV) hypertrophy appears with sustained hypertension, and is a predictor of cardiovascular morbidity and mortality in patients with hypertension. The regression of LV hypertrophy by means of antihypertensive treatment is associated with improvement in the prognoses of patients with hypertension (*1*, *2*). LV hypertrophy initially occurs as a compensatory response to pressure overload, but gradually changes to induce inadequate remodeling, finally leading to cardiac dysfunction, including systolic and diastolic heart failure (*3*, *4*). LV hypertrophy is characterized by hypertrophy of the cardiomyocytes and LV fibrosis and increased deposition of extracellular matrix (ECM) including collagen. The disproportionate synthesis and degradation of ECM plays an important role in the transition from LV hypertrophy to LV dysfunction (*5*, *6*). ECM synthesis is mainly regulated by myofibroblasts, which secrete ECM molecules in response to various cytokines and growth factors including transforming

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growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) (*7*, *8*). Together with these factors that regulate ECM synthesis, matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMP) also participate in the regulation of ECM remodeling (*6*, *9*, *10*). It has also been reported that the renin-angiotensin-aldosterone system has an important role in the upstream of these pathways for ECM regulation (*11–13*).

A peroxisome proliferator–activated receptor (PPAR) γ, a member of the nuclear receptor superfamily of ligand-activated transcription, plays a crucial role in adipogenesis and insulin resistance. A PPAR γ is highly expressed in adipose tissue, but several recent studies have shown that PPAR γ exists in myocytes, vascular smooth muscle cells, and macrophages/monocytes, as well as in adipocytes (*14*, *15*) Thiazolidinediones, which are PPAR γ activators, improve insulin sensitivity and are used as an anti-diabetic drug. This category of drug has also been shown to exert anti-inflammatory and anti-fibrotic actions in animal models of cardiovascular diseases, including atherosclerosis, vascular inflammation, and cardiac failure (*14–16*). The mechanisms underlying these actions are explained in part by insulin sensitization or PPAR γ–activation induced by these drugs. However, information is limited regarding the effects of thiazolidinediones on LV fibrosis. In addition, no study has examined cardiovascular organ damage in salt-sensitive hypertension. Dahl salt-sensitive (DS) rats, a model for salt-sensitive hypertension, develop severe hypertension and exhibit hypertensive target organ damage, such as cardiac hypertrophy and cardiac failure (*17*, *18*). Volume retention, the local renin-angiotensin system, oxidative stress, and tissue inflammation have been suggested as mechanisms of LV failure in this model (*19–23*). In the present study, we investigated whether or not pioglitazone, a thiazolidinedione, has beneficial effects on LV hypertrophy and fibrosis in salt-loaded DS rats, then compared its effects to those of candesartan, an angiotensin II receptor blocker.

Methods

Experimental Animals and Treatment

All experimental procedures were performed according to the National Institutes of Health guidelines for the care and use of laboratory animals. This experiment was approved by the Animal Care and Use Committee, University of the Ryukyus. Male DS rats were purchased from Seac Yoshitomi (Fukuoka, Japan). Rats (6 weeks old) were assigned randomly to four groups: a low-salt group (fed 0.3% NaCl rat chow; DSL, $n=6$), a high-salt group (fed 8% NaCl rat chow; DSH, $n=8$), a pioglitazone group (fed 8% NaCl rat chow and pioglitazone; DSH/pio, $n=8$), and a candesartan group (fed 8% NaCl rat chow and candesartan; DSH/can, $n=8$). Pioglitazone (10 mg/kg/day) was administered orally by mixing with high-salt rat chow. Candesartan (4 mg/kg/day) was administered by dissolving it in drinking water. To adjust the doses, the amount of drug mixed in the chow or in the drinking water was calculated from the amount of chow or water consumed on the preceding day. The drugs were administered from 6 weeks to 16 weeks.

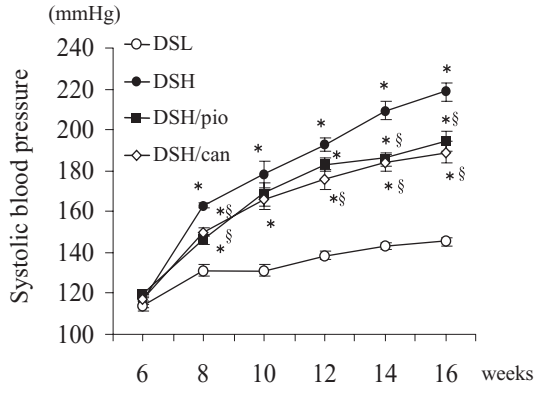

Fig. 1. *Time course changes in systolic blood pressure measured by the tail-cuff method in Dahl salt-sensitive (DS) rats. Data represent means±SEM. DSL, DS rats fed with low salt ($n=6$); DSH, DS rats fed with high salt ($n=8$); DSH/pio, DSH treated with pioglitazone ($n=8$); DSH/can, DSH treated with candesartan ($n=8$). *$p<0.05$ vs. DSL, §$p<0.05$ vs. DSH.*

Blood Pressure Measurement and Sample Collection

Systolic blood pressure was measured by the noninvasive tail-cuff system (MK-2000, Muromachi Kikai, Tokyo, Japan) every 2 weeks throughout the experimental period. At 16 weeks of age, the rats were sacrificed under deep anesthesia with an intraperitoneal injection of sodium pentobarbital. Blood samples were collected for the measurement of plasma glucose and insulin, and then the heart and the lung tissues were removed. The heart weight (HW) and the wet lung weight (LW) were determined and normalized by body weight (BW). LV tissue was frozen immediately in liquid nitrogen and stored at −80°C until use. Plasma insulin and glucose were measured by enzyme assay and enzyme immunoassay, respectively.

The other rats were sacrificed with a sodium pentobarbital overdose and were perfusion-fixed *via* apex for 5 min at 200 mmHg in DSH, 180 mmHg in DSH/pio and DSH/can, and 140 mmHg in DSL with 4% paraformaldehyde buffered with 0.1 mol/L NaH_2PO_4. The heart was then excised and processed in paraffin using routine techniques.

Echocardiography

At 16 weeks of age, the rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection). Transtho-

Table 1. Body Weight, Weight of Target Organ in DS Rats at 16 Weeks of Age

	Treatment group			
	DSL	DSH	DSH/pio	DSL/can
BW (g)	403±7	369±8*	383±3*	379±4*
HW (g)	1.50±0.02	1.82±0.02*	1.78±0.03*	1.72±0.02*,§
HW/BW (mg/g)	3.7±0.1	4.9±0.1*	4.7±0.1*,§	4.5±0.0*,§
LW (g)	2.05±0.08	2.32±0.11	2.07±0.15	1.92±0.08§
LW/BW (mg/g)	5.1±0.2	6.3±0.3*	5.4±0.4§	5.1±0.2§

Values are means±SEM, $n=5$–6 per group. Treatment group: DSL, low-salt group; DSH, high-salt group; DSH/pio, pioglitazone group; DSH/can, candesartan group. DS rats, Dahl salt-sensitive rats; BW, body weight; HW, heart weight; LW, lung weight. *$p<0.05$ *vs*. DSL, §$p<0.05$ *vs*. DSH.

Table 2. Transthoracic Echocardiography Measurements in DS Rats at 16 Weeks of Age

	Treatment group			
	DSL	DSH	DSH/pio	DSL/can
IVS (mm)	1.9±0.1	2.2±0.1*	1.9±0.0§	1.9±0.0§
PW (mm)	1.8±0.0	2.1±0.1*	1.9±0.0§	1.9±0.0§
RWT	0.35±0.01	0.38±0.00*	0.33±0.01§	0.34±0.01§
LVDs (mm)	3.8±0.16	3.9±0.16	4.3±0.04*,§	3.8±0.01
LVDd (mm)	7.0±0.15	7.1±0.20	7.5±0.11*,§	7.3±0.09
EF (%)	70.9±2.2	69.5±1.9	68.2±0.5	72.9±1.3
%FS (%)	46.2±2.1	45.0±1.8	43.6±0.5	48±1.2

Values are means±SEM, $n=6$ per group. Treatment group: DSL, low-salt group; DSH, high-salt group; DSH/pio, pioglitazone group; DSH/can, candesartan group. DS rats, Dahl salt-sensitive rats; IVS, interventricular septal wall thickness; PW, posterior wall thickness; RWT, relative wall thickness; LVDs, left ventricular end-systolic dimension; LVDd, left ventricular end-diastolic dimension; EF, ejection fraction; %FS, percent fractional shortening. *$p<0.05$ *vs*. DSL, §$p<0.05$ *vs*. DSH.

racic echocardiography (Sonolayer, Toshiba, Tokyo, Japan; 7.5 MHz linear-array transducer) was performed by one of the authors (M.N.). Interventricular septal wall thickness (IVS), LV posterior wall thickness (PW), LV end-diastolic dimension (LVDd), and LV end-systolic dimension (LVDs) were determined. Percent fractional shortening (%FS) and ejection fraction (EF) of LV were calculated as described elsewhere. Relative wall thickness (RWT) was calculated as RWT = (IVS + PW)/LVDd.

Histological Examination

LV tissues from the LV midcavity were sliced 3 μm thick. The sections were then deparaffinized and dehydrated, and each type of staining was performed. Masson's trichrome staining, hematoxylin-eosin (H-E) staining, and α-smooth muscle actin by immunohistochemical staining were used for the analysis of cardiac interstitial and perivascular fibrosis, cardiac myocyte area, and the appearance of cardiac myofibroblast, respectively.

The entire section was quantified with the use of image analysis software (Image Pro-Plus, Silver Spring, USA). To determine interstitial fibrosis, a total of 30 areas were randomly selected per animal from the free wall and the septum of the LV, and the areas of fibrosis and myocytes was calculated and used for the analysis. To evaluate perivascular fibrosis, approximately 20 vasculatures were randomly selected from each animal, and the area of fibrosis was measured for each animal. To assess cross-sectional areas, cardiomyocytes, which were cut transversely and had nuclei in their centers, were selected from random fields in each analyzed tissue. Nearly 100 myocytes were measured for each animal and used in the analysis.

The α-smooth muscle actin staining was performed with mouse anti–actin smooth muscle monoclonal antibody (10:1 dilution, Nichirei Bioscience, Tokyo, Japan) by the conventional method.

Quantitative Real-Time Polymerase Chain Reaction

For quantitative polymerase chain reaction (PCR), total RNA was extracted from LV tissue by using ISOGEN (Nippon Gene, Tokyo, Japan). Reverse transcription of 5 μg of total RNA was performed with the use of High Fidelity RNA PCR kit (Takara Bio, Otsu, Japan). PCR reactions were performed with the LightCycler FastStart DNA Master SYBR Green I and LightCycler (Roche Diagnostics, Mannheim, Germany).

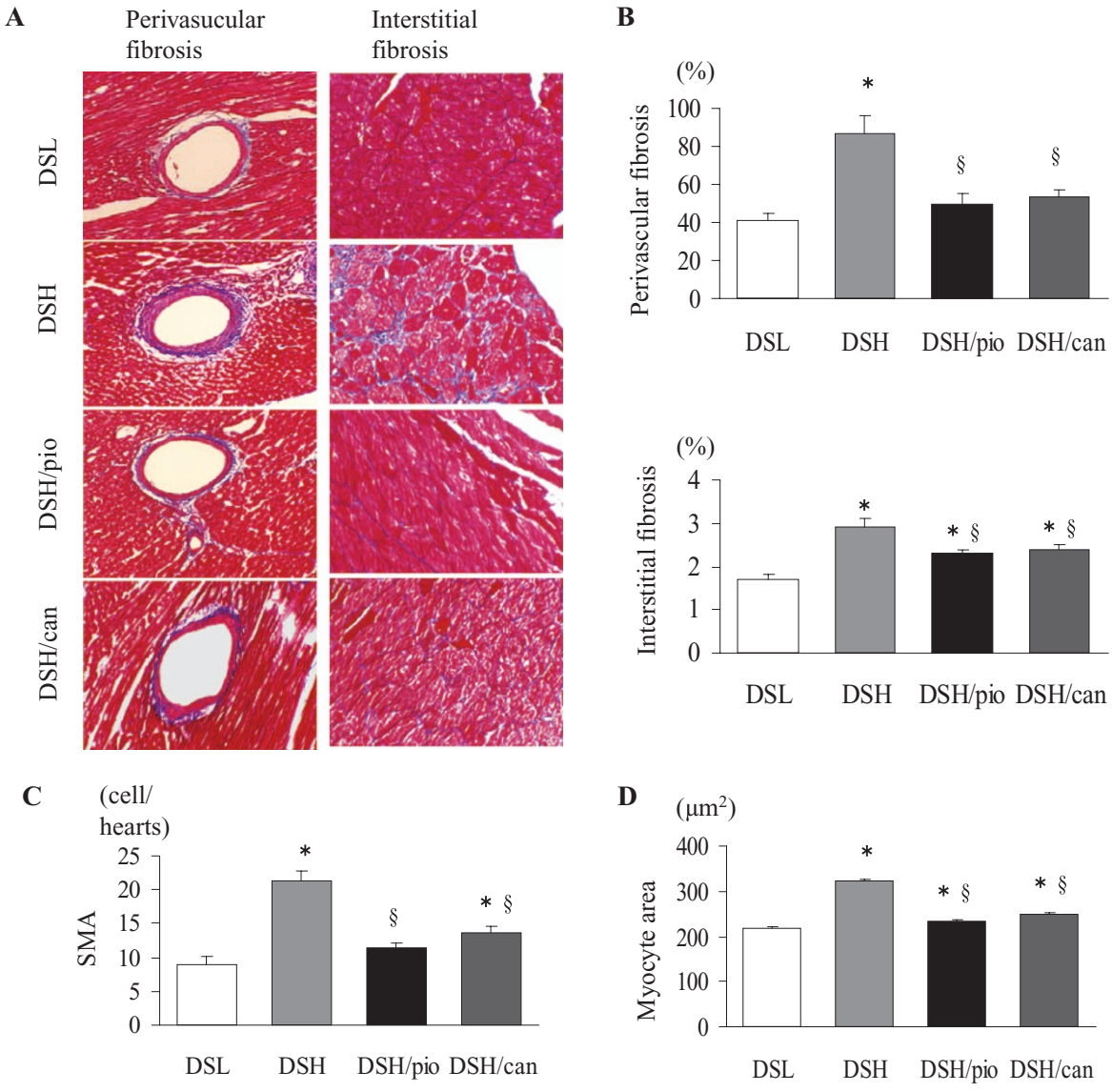

Fig. 2. *Histological examination of the left ventricle (LV) in DS rats at 16 weeks of age. A: Representative photomicrographs of interstitial and perivascular fibrosis in LV sections by Masson's trichrome staining. Blue staining demonstrates collagen deposition. B: Percentages of interstitial and perivascular areas by Masson's trichrome staining. C: Number of myofibroblasts in the LV evaluated by immunochemical staining for SMA. D: Myocyte cross-sectional areas in the LV. Data represent means±SEM. n=3–4 per group. *p<0.05 vs. DSL, §p<0.05 vs. DSH. SMA, α smooth muscle actin.*

The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), brain natriuretic peptide (BNP), types I and III collagen, TIMP 1 and 2, and TGF-β1 were quantified. GAPDH served as an internal control.

Zymography

Gelatin zymography was performed to evaluate the gelatinase activity of LV tissue as described elsewhere (*11*, *12*). The MMP activities were determined by the proteolytic levels and quantified with the use of NIH Image software.

Measurement of Thiobarbituric Acid–Reactive Substances (TBARS) Level

We evaluated the degree of lipid peroxidation as a marker of oxidative stress. We measured the thiobarbituric acid–reactive substances (TBARS) of cardiac tissues after homogenization and plasma by using the OXI-tek TBARS assay kit (ZeptoMetrix, Buffalo, USA). The TBARS level of cardiac tissues was corrected by protein level.

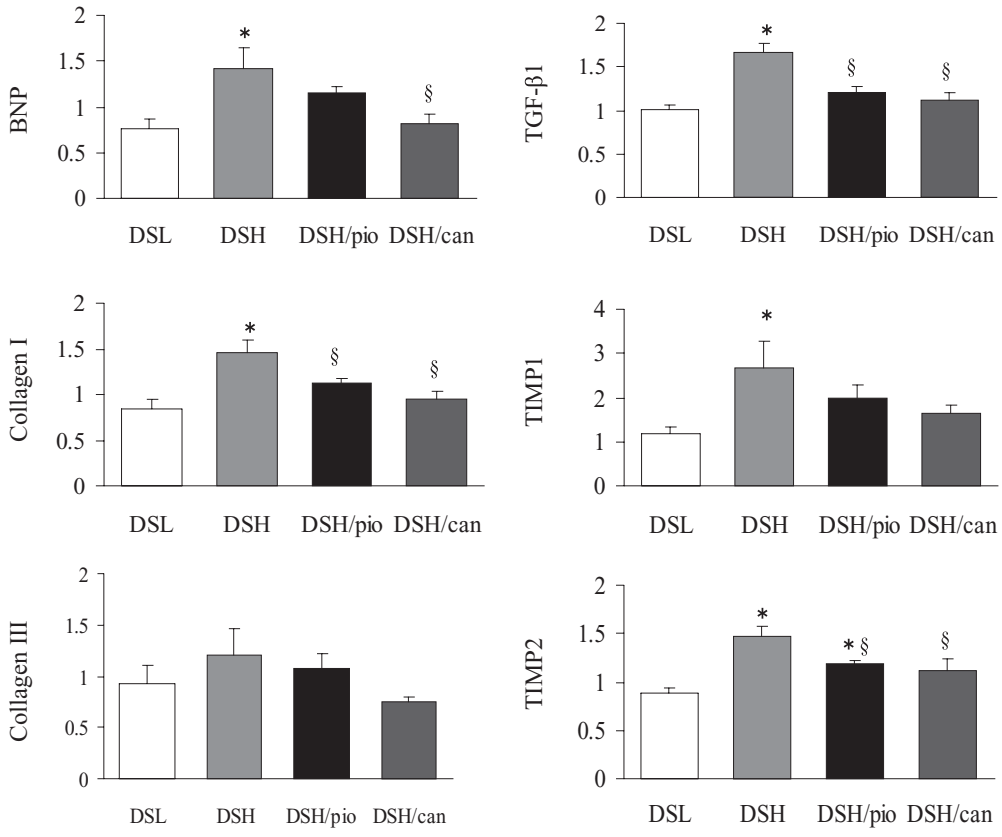

Fig. 3. *Quantitative real-time polymerase chain reaction measurement of expressions of mRNA in the LV. The expression level of each sample was expressed relative to that of glyceraldehyde-3-phosphate dehydrogenase, and is presented as a relative value with the DSL used for comparison. Data represent means±SEM, n=6–8 per group. *p<0.05 vs. DSL, §p<0.05 vs. DSH. BNP, brain natriuretic peptide; TIMP, tissue inhibitors of matrix metalloproteinase; TGF-β1, transforming growth factor-β1.*

Statistical Analysis

Values are expressed as means±SEM. Differences among the groups were tested by analysis of variance with or without repeated measures. Subsequent analysis for significant differences was performed using Fisher's *F* test. A value of $p<0.05$ was considered significant.

Results

Systolic Blood Pressure and Organ Weights

As shown in Fig. 1, systolic blood pressure was steadily elevated with a high-salt diet. Both candesartan and pioglitazone significantly decreased blood pressure, although the blood pressure levels in DSH/can and DSH/pio remained higher than in DSL (systolic blood pressure at 16 weeks of age: DSL 145±2 mmHg, DSH 216±4 mmHg, DSH/pio 195±5 mmHg, and DSH/can 189±5 mmHg).

BW was lower in DSH, DSH/pio, and DSH/can than in DSL (Table 1). HW and HW/BW were higher in DSH than in DSL. Candesartan reduced the elevation of HW, but pioglitazone did not, whereas both drugs decreased HW/BW. Furthermore, LW or LW/BW, either of which could reflect pulmonary congestion, was greater in DSH than in DSL. Candesartan normalized both LW and LW/BW, whereas pioglitazone decreased LV/BW without altering LW. The LW/BW of DSH/can was comparable to that of DSH/pio.

Concentrations of Plasma Glucose and Insulin

Pioglitazone and candesartan did not apparently affect the plasma glucose level (DSH: 99±5 mg/dL; DSH/pio: 85±3 mg/dL; DSH/can: 83±3 mg/dL; $n=5$–6 per group), but significantly decreased the plasma insulin level (DSH: 1.0±0.2 ng/mL; DSH/pio: 0.6±0.1 ng/mL; DSH/can 0.7±0.1 ng/mL; $n=5$–6 per group, $p<0.05$ for both treatments *vs*. DSH).

Echocardiography

IVS, PW, and RWT were greater in DSH than in DSL (Table 2). Both candesartan and pioglitazone nearly normalized the

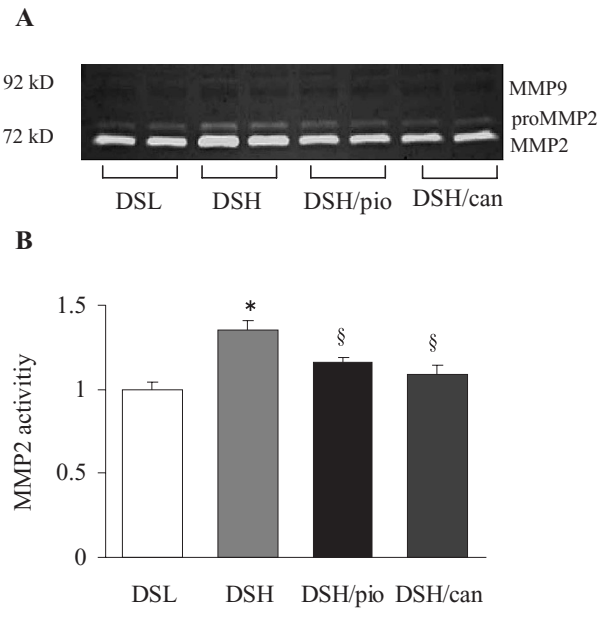

Fig. 4. *Matrix metalloproteinase (MMP) activities in the LV. A: Representative gelatin zymography of LV tissue. B: Quantitative analysis of MMP2 activity of zymography in the LV. The activity level of each sample was presented as a relative value with the DSL used for comparison. Data represent means±SEM, n=6 per group. *p<0.05 vs. DSL, §p<0.05 vs. DSH.*

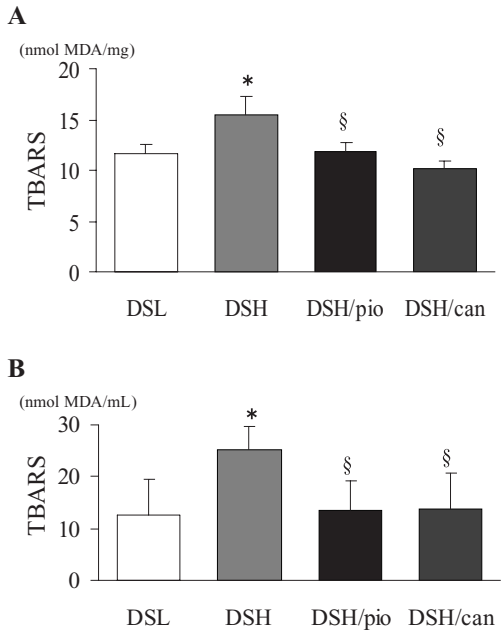

Fig. 5. *Levels of thiobarbituric acid–reactive substances (TBARS) in the LV (A) and plasma (B). Data represent means±SEM, n=5–6 per group. *p<0.05 vs. DSL, §p<0.05 vs. DSH. MDA, malon-dialdehyde.*

increased LV wall thickness. Pioglitazone slightly increased LVDd and LVDs, but candesartan did not. Pioglitazone did not apparently affect EF or %FS; candesartan, however, tended to increase these values.

Cardiac Fibrosis and Cardiomyocyte Hypertrophy

Masson's trichrome staining showed that interstitial and perivascular fibroses were more evident in DSH than in DSL (Fig. 2). Both pioglitazone and candesartan significantly decreased interstitial and perivascular fibrosis in DSH. We also evaluated the number of α smooth muscle actin–positive fibroblasts, namely cardiac myofibroblasts, in the LV. The number of myofibroblasts was greater in DSH than in DSL (Fig. 2). Pioglitazone and candesartan decreased the numbers of myofibroblasts in LV tissue.

Myocardial cells were larger in DSH than in DSL (Fig. 2). Pioglitazone and candesartan significantly reduced cell size. Cell size was comparable between DSH/pio and DSH/can.

Gene Expression

We investigated the expression levels of genes reportedly associated with LV hypertrophy and ECM remodeling. The expression levels of BNP, collagen type I, TIMP1 and 2, and TGF-β1 were significantly greater in DSH than in DSL (Fig. 3). No difference was observed between DSH and DSL in the expression of collagen type III mRNA. Candesartan significantly decreased the elevation of the levels of mRNA in BNP, collagen type I, TIMP2, and TGF-β1. Pioglitazone also decreased the mRNA levels in collagen type I, TGF-β1, and TIMP2. However, the decrease in TIMP1 induced by candesartan and that in BNP induced by pioglitazone did not reach the level of significance.

Zymography

MMP activity has been shown to be associated with the transition of LV hypertrophy to LV failure in salt-loaded DS rats (*11*, *12*). To determine the change in MMP activity, the gelatinolytic activity of LV was assessed. The MMP2 level was significantly higher in DSH than in DSL (Fig. 4). Both pioglitazone and candesartan significantly decreased MMP2 activity. In contrast, the correction of MMP9 activity could not be evaluated because of its low level of expression or activity.

TBARS Analysis

The TBARS level of LV tissues was significantly higher in DSH than in DSL (Fig. 5). Pioglitazone and candesartan significantly decreased the TBARS level in LV tissues to a similar extent. Pioglitazone and candesartan also decreased the plasma TBARS level in DSH (Fig. 5).

Discussion

The present study demonstrated that pioglitazone ameliorated LV hypertrophy and fibrosis in salt-loaded DS rats. Although the precise mechanisms underlying these actions were not determined in the present study, various factors, including decreases in blood pressure, insulin level, and oxidative stress, could be associated with the changes in the LV.

Pioglitazone significantly decreased the blood pressure of salt-loaded DS rats in the present study. A decrease in blood pressure could contribute to the regression of LV hypertrophy. The antihypertensive actions of thiazolidinediones in hypertensive model animals were inconsistent among previous papers (*24–29*). Chronic administration of thiazolidinediones decreased blood pressure in deoxycorticosterone acetate (DOCA)–salt rats, angiotensin II–induced hypertensive rats, and SHR (*24*, *26–28*), but had no or only a slight effect on blood pressure in L-NAME–induced hypertensive rats (*29*) and adult stroke-prone SHR (our unpublished observation). On the other hand, thiazolidinediones decreased blood pressure in model animals with diabetes or insulin resistance (*30*, *31*). Since DS rats are known to have insulin resistance (*32*, *33*), the antihypertensive action may be explained in part by its insulin-sensitizing action. In our study, pioglitazone administration decreased the plasma insulin level, which may be associated with the improvement of insulin resistance, although we did not evaluate insulin resistance by either a glucose-tolerance test or an insulin-infusion test in the present study. Besides their insulin-sensitizing action, thiazolidinediones exhibit direct vascular protection by inhibiting tissue inflammation, modulating the growth and proliferation of vascular smooth muscle cells, and improving endothelial cell function (*14*, *15*, *25*, *28*, *34*). These mechanisms confer the antihypertensive action of this drug.

The renin-angiotensin system plays an important role in the pathogenesis of LV remodeling and the transition from LV hypertrophy to LV dysfunction in salt-sensitive hypertension (*11*, *12*). Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers corrected LV hypertrophy and prevented LV failure (*11*, *12*, *23*). The present study showed that candesartan improved LV hypertrophy and fibrosis in salt-loaded DS rats. This beneficial action by candesartan could be due to the inhibition of angiotensin II type 1 receptor (*23*, *35*). We also showed that pioglitazone exerted similar anti-hypertrophic and anti-fibrotic actions on the heart. Only a few studies have examined effects of thiazolidinediones on LV hypertrophy; rosiglitazone did not affect cardiac hypertrophy in DOCA-salt rats (*26*, *27*).

Hypertensive LV hypertrophy is characterized by hypertrophy of cardiomyocytes and tissue fibrosis, specifically increased deposition of ECM including collagen. ECM generation was mainly regulated by the proliferation of fibroblasts and the transition to myofibroblasts, which were stimulated by angiotensin II, TGF-β, and CTGF (*7*, *8*). A high-salt diet also facilitated cardiac fibrosis with enhanced expression of TGF-β in both WKY and SHR (*36*). The production of reactive oxygen species *via* nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was also involved in the activation of cardiac myofibroblasts (*37*). In the present study, the high-salt diet increased interstitial and perivascular fibrosis along with enhanced expression of TGF-β and production of reactive oxygen species in LV in DS rats. These effects were ameliorated by candesartan and pioglitazone.

Since both candesartan and pioglitazone decreased the number of myofibroblasts, the gene expression of TGF-β, and the reactive oxygen species level in the LV, it is possible that both drugs may affect the same pathway of LV fibrosis. In a recent study by Chen *et al*., pioglitazone attenuated the angiotensin II–mediated collagen type I synthesis in cultured cardiac fibroblasts (*38*). Also in that study, this effect of pioglitazone was mediated by the inhibition of reactive oxygen species production and redox-sensitive transcription factors. Another possibility is that pioglitazone may affect angiotensin receptor expression. Previous studies have reported that PPAR γ activators down-regulate the expression of the angiotensin II type 1 receptor (*39*, *40*). In another study, no modulation of the angiotensin II receptor by thiazolidinedione was observed (*41*). In our preliminary study, the mRNA levels of angiotensin II receptor in the LV from control DSH were comparable to those in the LV from pioglitazone-treated DSH (data not shown).

Insulin is one of the growth factors that stimulate the proliferation and hypertrophy of various cells, including cardiomyocytes and fibroblasts (*42*). Insulin stimulates cardiomyocyte hypertrophy through the phosphatidyl inositol-3 kinase (PI3K)/Akt-1 pathway, the extracellular signal–regulated kinase (ERK)/mitogen-activated protein (MAP) kinase pathway, and Rho/Ras-associated pathways. Insulin also stimulates sympathetic nervous activity, which could cause LV hypertrophy as well as hypertension. Thus, an improvement in insulin resistance and/or a decrease in insulin level *per se* may contribute to the regression of cardiac cell hypertrophy.

Although pioglitazone and candesartan improved LV hypertrophy and fibrosis, their actions on LV geometry were not the same in the present study. Pioglitazone decreased HW/BW and LV wall thickness while increasing LV diameter, as shown in echocardiograms. In contrast, candesartan decreased HW, HW/BW, and LV wall thickness without changing LV diameter. One may be concerned that pioglitazone deteriorated the LV systolic function and facilitated the transition from concentric hypertrophy to eccentric remodeling in the present study. However, this is unlikely, for the following reasons. First, pioglitazone did not significantly affect EF or %FS. Second, pioglitazone tended to decrease lung weight (a marker of lung congestion) and the mRNA level of BNP (a marker of LV dysfunction). Third, pioglitazone decreased MMP2 activity, which is considered a marker of the transition from LV hypertrophy to LV failure in

Dahl rats (*11*, *12*).

It has been reported that cardiomyocyte-specific PPAR γ knockout mice develop cardiac hypertrophy as a result of increased nuclear factor κB activity (*43*). This suggests that PPAR γ suppresses LV hypertrophy. In previous studies, pioglitazone suppressed cardiac hypertrophy in response to agonists or mechanical stimulation (*44*, *45*). In contrast, rosiglitazone stimulated cardiac hypertrophy in mice *via* a PPAR γ–independent mechanism (*43*). Although the actions of PPAR γ agonists on the heart are not fully understood, PPAR γ plays a crucial role in the pathway regulating cardiac hypertrophy.

Thiazolidinediones expand body fluid volume (*46*, *47*). The mechanism underlying this action has not been fully elucidated, but one possibility is that PPAR γ mediated renal salt abortion *via* the epithelial Na^+ channel (*46*). This volume retention may be associated with the pioglitazone-induced LV enlargement in the present study, because BW increased (though only slightly) with pioglitazone administration.

In summary, pioglitazone and candesartan ameliorated LV hypertrophy and fibrosis in DS rats fed a high-salt diet. We may hypothesize from these results that thiazolidinediones are beneficial for LV hypertrophy in human hypertension. This hypothesis will be tested in future clinical studies. In the meantime, caution should be taken because thiazolidinediones may cause fluid retention and worsen congestive heart failure (*47*).

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