**Abstract**

**Objectives:** Oxidative stress is implicated in the pathogenesis of heart failure and affects the activity of matrix metalloproteinases (MMPs). We have now investigated the role of MMPs and their tissue inhibitors (TIMPs) in the transition from compensated left ventricular (LV) hypertrophy to heart failure as well as the effects of pravastatin on this transition in a rat model.

**Methods:** Dahl salt-sensitive rats were fed a high-salt (8% NaCl) diet and treated with pravastatin (50 or 100 mg/kg per day) or vehicle from 7 weeks of age.

**Results:** Pravastatin did not attenuate LV hypertrophy apparent at 12 or 18 weeks of age. However, the high dose of this drug markedly improved indices of diastolic function (early diastolic myocardial velocity) and systolic function (LV fractional shortening) at 18 weeks of age and increased the survival rate. It also prevented a decrease in the ratio of reduced to oxidized glutathione and an increase in NADPH oxidase activity in the left ventricle induced by the high-salt diet. The activities of MMP2 and MMP9 and the abundance of TIMP1 and TIMP2 in LV tissue were increased at 18 weeks of age, and pravastatin also prevented these changes.

**Conclusion:** Although pravastatin did not attenuate LV hypertrophy, it prevented the transition from compensated hypertrophy to heart failure in this rat model. This effect of pravastatin may result from a reduction both in the level of oxidative stress and in MMP activity in the heart.

© 2005 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

**Keywords:** Heart failure; Matrix metalloproteinases; NAD(P)H-oxidase; Oxidative stress; Statins

---

**1. Introduction**

Reactive oxygen species (ROS) are implicated in cardiovascular conditions such as atherosclerosis, reperfusion injury, hypertension, and heart failure [1,2]. An increase in oxidative stress resulting from increased cardiac generation of ROS is thought to contribute to contractile and endothelial dysfunction, myocyte apoptosis and necrosis, and remodeling of the extracellular matrix in the heart [3]. Such oxidative stress has indeed been demonstrated in patients with various hypertensive disorders [4] as well as in animal models of heart failure [5].

ROS react with thiol groups, which are important in preservation of matrix metalloproteinase (MMP) latency and have been shown to affect the activity of MMPs [6]. MMPs belong to a family of proteolytic enzymes that are thought to play a major role in degradation of extracellular proteins in the myocardium. Activation of MMPs has been
detected in the failing human heart [7], and changes in MMP levels in the myocardium were also accompanied by alterations in extracellular matrix structure. Tissue inhibitors of MMPs (TIMPs) are locally synthesized proteins that bind to active MMPs and thereby inhibit their proteolytic activity. A balance between the concentrations of MMPs and TIMPs exists in the normal myocardium, and changes in this balance have been implicated in progression of ventricular dilation and the development of heart failure [8,9].

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, have become the most widely administered agents to reduce serum lipid concentrations and the associated risk for cardiovascular disease [10]. Evidence also suggests that statin therapy prevents alterations and the associated risk for cardiovascular disease [11]. Additional beneficial cardiovascular effects of statins include protection of the bioactivity of endothelial nitric oxide, inhibition of the secretion of MMPs by macrophages, inhibition of the expression of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, and attenuation of the formation of superoxide [14–16].

We have now investigated whether pravastatin prevents myocardial hypertrophy or the progression from compensated left ventricular (LV) hypertrophy to heart failure in Dahl salt-sensitive (DS) rats. We also examined the effects of this drug on changes in oxidative stress and MMP activity in the heart found to accompany heart failure in this animal model.

2. Methods

2.1. Animals

Male inbred DS rats (Eisai, Tokyo, Japan) were fed a low-salt (0.3% NaCl) diet from weaning until 7 weeks of age. Thereafter, they were fed a high-salt (8% NaCl) diet and randomly divided into three groups: those treated with vehicle (8% NaCl group), those treated with a low dose (50 mg/kg of body weight per day) of pravastatin (8% NaCl+Pra50 group), and those treated with a high dose (100 mg/kg per day) of pravastatin (8% NaCl+Pra100 group). Pravastatin (Sankyo, Tokyo, Japan) or vehicle (5% carboxymethylcellulose sodium) was administered orally by gastric gavage once a day until 18 weeks of age. DS rats maintained on a diet containing 0.3% NaCl until 18 weeks of age served as controls. The experimental diet had contents of protein, minerals, and fat similar to those of standard chow (MF rat diet; Oriental Yeast, Tokyo, Japan). All experimental procedures were performed in accordance with institutional guidelines for animal research. The investigation also conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Physiological measurements and determination of serum lipid concentrations

Systolic blood pressure of conscious rats was measured by the tail-cuff method. At 12 and 18 weeks of age, transthoracic echocardiographic analyses were performed with a SONOS 5500 system (Philips Medical Systems, Bothell, WA) as previously described [17]. Wall thickness and LV end-diastolic diameter (LVDd) were obtained from a short-axis view at the level of the papillary muscles, and LV fractional shortening was calculated. The early diastolic myocardial velocity (MV) was derived from tissue Doppler imaging of the epicardium for evaluation of diastolic function [18].

At 12 and 18 weeks of age, after physiological measurements, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and arterial blood was immediately collected from the abdominal aorta. Serum was isolated and assayed for total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides by enzymatic methods [19].

2.3. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The left ventricle was separated from the atria and the right ventricle, weighed, and immediately frozen in liquid nitrogen. Total RNA was extracted from LV tissue and subjected to quantitative RT-PCR analysis as described [20] with primers specific for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (βMHC), collagen I, collagen III, fibronectin, MMP2, MMP9, TIMP1, and TIMP2 genes. TaqMan rodent glycerolalddehyde-3-phosphate dehydrogenase (GAPDH) control reagents (Perkin-Elmer, Wellesley, MA) were used for the detection of GAPDH mRNA as an internal standard.

2.4. Morphological measurements

The left ventricle was fixed with 4% paraformaldehyde, embedded in paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin–eosin or Azan Mallory solution. The cross-sectional area of myocytes and the extents of interstitial and perivascular fibrosis were determined as described [17].

2.5. Assay of glutathione and NADPH oxidase activity

The amount of total glutathione [reduced (GSH) and oxidized (GSSG)] in the left ventricle was determined by the glutathione reductase and 5,5'-dithiobis-(2-nitrobenzoic acid) recycling assay as described [21]. The amount of GSSG was determined by Griffith’s method [22]. Specific myocardial NADPH oxidase activity was measured in total homogenates of the left ventricle with the use of a lucigenin-based enhanced chemiluminescence assay as described [23]. The chemiluminescence signal was sampled every minute.
for 10 min with a luminescence reader (BLR-201; Aloka, Tokyo, Japan), and the respective background counts were subtracted from experimental values. Luciferin chemiluminescence was expressed as counts per minute per milligram of protein.

2.6. Immunoblot analysis

Total membrane and cytosolic fractions were isolated from LV tissue and subjected to immunoblot analysis with rabbit polyclonal antibodies to H-Ras or to Rob1 (1:200 dilutions; Santa Cruz Biotechnology, Santa Monica, CA) or with mouse monoclonal antibodies to Rac1 (1:1000 dilution; Transduction Laboratories, Lexington, KY) or to p47phox or p67phox subunits of NADPH oxidase (1:500 dilutions; Transduction Laboratories). Immune complexes were detected with appropriate horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Piscataway, NJ) and quantitated with Quantity One Image software (Bio-Rad, Hercules, CA).

2.7. Assay of cardiac MMP activity and TIMP abundance

The activities of MMP2 and MMP9 in total homogenates of LV tissue were measured with an antibody-capture method (Amersham Biosciences) as described [24]. MMP activity was expressed as nanograms of substrate hydrolyzed per hour per gram of homogenate protein. The total abundance (free and MMP-complexed forms) of TIMP1 (Amersham Biosciences) and TIMP2 (R and D Systems, Minneapolis, MN) in homogenates of LV tissue was determined with enzyme-linked immunosorbent assay kits as described [25]. Data are expressed as picograms of TIMP per milligram of homogenate protein.

2.8. Statistical analysis

Data are presented as means±SEM. Paired data were analyzed by the paired Student’s t test. Differences in multiple parameters between two groups were evaluated by two-way analysis of variance (ANOVA), and significant differences were compared by one-way ANOVA followed by Dunnett’s post hoc test. Survival rate was analyzed by the standard Kaplan–Meier method with a log-rank test. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Systolic blood pressure and serum lipid levels

DS rats fed a high-salt diet from 7 weeks of age progressively developed hypertension. Systolic blood pressure was significantly higher in all animals on the 8% NaCl diet than in those on the 0.3% NaCl diet from 8 to 18 weeks of age (Table 1). Treatment with pravastatin at the low or

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3% NaCl</th>
<th>8% NaCl</th>
<th>8% NaCl+Pra50</th>
<th>8% NaCl+Pra100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>385.8±9.5</td>
<td>439.9±2.8</td>
<td>364.7±12.7</td>
<td>328.4±4.6†</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>170.9±1.7</td>
<td>169.9±1.8</td>
<td>244.7±0.8*</td>
<td>247.2±3.7†</td>
</tr>
<tr>
<td>T-chol (mg/dL)</td>
<td>65.8±2.31</td>
<td>80.0±3.73</td>
<td>99.0±4.68*</td>
<td>131.2±7.49†</td>
</tr>
<tr>
<td>HDL-chol (mg/dL)</td>
<td>31.3±1.11</td>
<td>31.8±1.28</td>
<td>36.1±1.35*</td>
<td>38.5±2.47†</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>141.5±13.8</td>
<td>190.5±16.3</td>
<td>124.2±2.60</td>
<td>148.3±3.76</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>385.8±9.5</td>
<td>439.9±2.8</td>
<td>364.7±12.7</td>
<td>328.4±4.6†</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>170.9±1.7</td>
<td>169.9±1.8</td>
<td>244.7±0.8*</td>
<td>247.2±3.7†</td>
</tr>
<tr>
<td>T-chol (mg/dL)</td>
<td>65.8±2.31</td>
<td>80.0±3.73</td>
<td>99.0±4.68*</td>
<td>131.2±7.49†</td>
</tr>
<tr>
<td>HDL-chol (mg/dL)</td>
<td>31.3±1.11</td>
<td>31.8±1.28</td>
<td>36.1±1.35*</td>
<td>38.5±2.47†</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>141.5±13.8</td>
<td>190.5±16.3</td>
<td>124.2±2.60</td>
<td>148.3±3.76</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3% NaCl</th>
<th>8% NaCl</th>
<th>8% NaCl+Pra50</th>
<th>8% NaCl+Pra100</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS (mm)</td>
<td>1.56±0.04</td>
<td>1.84±0.04</td>
<td>2.64±0.12*</td>
<td>2.36±0.13†</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>1.64±0.05</td>
<td>1.83±0.06</td>
<td>2.59±0.11*</td>
<td>2.31±0.19†</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>0.87±0.03</td>
<td>0.99±0.03</td>
<td>1.49±0.07*</td>
<td>1.77±0.17†</td>
</tr>
<tr>
<td>LVM (g)/BW (kg)</td>
<td>2.27±0.05</td>
<td>2.25±0.06</td>
<td>4.11±0.17*</td>
<td>5.17±0.67†</td>
</tr>
<tr>
<td>LVDD (mm)</td>
<td>7.57±0.18</td>
<td>7.48±0.17</td>
<td>7.08±0.20</td>
<td>8.06±0.05†</td>
</tr>
<tr>
<td>FS (%)</td>
<td>43.01±0.80</td>
<td>42.96±1.24</td>
<td>45.00±1.63</td>
<td>28.1±1.63†</td>
</tr>
<tr>
<td>MV (cm/s)</td>
<td>3.73±0.15</td>
<td>3.72±0.28</td>
<td>2.73±0.14*</td>
<td>2.31±0.08†</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>1.43±0.04</td>
<td>1.43±0.04</td>
<td>3.63±1.49†</td>
<td>3.49±1.09†</td>
</tr>
</tbody>
</table>

IVS, interventricular septum thickness; PW, left ventricular posterior wall thickness; LVM, left ventricular mass; BW, body weight; LVDD, left ventricular end-diastolic diameter; FS, left ventricular fractional shortening; MV, myocardial velocity (early diastolic). Data are means±SEM of values from five rats in the 8% NaCl+Pra50 and 8% NaCl+Pra100 groups at 18 weeks and from six rats in all other groups. *P<0.05 vs. rats fed the 0.3% NaCl diet at 12 weeks; †P<0.05 vs. rats fed the 0.3% NaCl diet at 18 weeks; ‡P<0.05 vs. rats fed the 8% NaCl diet (without pravastatin) at 18 weeks.
high dose did not affect blood pressure. The serum concentrations of total cholesterol and HDL-cholesterol were significantly higher in all rats fed the 8% NaCl diet than in those on the 0.3% NaCl diet at both 12 and 18 weeks of age and were not affected by treatment with pravastatin (Table 1). The serum concentration of triglycerides did not differ among the four groups of rats.

3.2. Echocardiographic findings

Echocardiographic parameters at 12 and 18 weeks of age are shown in Table 2. At 12 weeks of age, cardiac hypertrophy as reflected by LV wall thickness and mass was apparent in all rats fed the high-salt diet and was not affected by treatment with pravastatin. At 18 weeks of age, although there was still no difference in LV wall thickness or mass among the three groups of rats on the 8% NaCl diet, the LVDd was significantly greater in the 8% NaCl group than in the 0.3% NaCl group but was significantly smaller in the 8% NaCl + Pra100 group than in the 8% NaCl group. The early diastolic MV and LV fractional shortening were significantly smaller in the 8% NaCl group than in the 0.3% NaCl group at 18 weeks of age, but these parameters were significantly greater in the 8% NaCl + Pra100 group than in the 8% NaCl group at this time. Pravastatin at the high dose thus improved diastolic
and systolic function in animals on the high-salt diet at 18 weeks of age.

3.3. Survival rate

Kaplan–Meier analysis revealed that the survival rate of rats in the 8% NaCl group was markedly reduced compared with that of animals in the 0.3% NaCl group (Fig. 1). However, the survival rate was significantly greater in the 8% NaCl + Pra100 group than in the 8% NaCl or 8% NaCl + Pra50 groups.

3.4. Cardiac phenotype-related gene expression

The abundance of βMHC and ANP mRNAs in the left ventricle was significantly greater in the 8% NaCl group.

---

Fig. 3. Morphological analysis of the left ventricle of DS rats at 18 weeks of age. (A) Light micrographs of myocytes in hematoxylin–eosin-stained sections of the LV wall of rats in the 0.3% NaCl (a), 8% NaCl (b), 8% NaCl + Pra50 (c), and 8% NaCl + Pra100 (d) groups are shown on the left. Magnification, ×400. The cross-sectional area of myocytes determined from such micrographs is shown on the right. (B and C) Light micrographs of sections of the left ventricle stained with Azan Mallory solution are shown on the left. Magnification, ×200. The extents of interstitial (B) and perivascular (C) fibrosis are shown on the right. All quantitative data are means ± SEM of values from six rats per group. *P < 0.05 vs. rats fed the 0.3% NaCl diet; †P < 0.05 vs. rats fed the 8% NaCl diet without pravastatin.
than in the 0.3% NaCl group at 18 weeks of age (Fig. 2). Although treatment with pravastatin tended to reduce the amounts of these mRNAs, these effects were not statistically significant. The abundance of BNP mRNA in the left ventricle was also significantly greater in the 8% NaCl group than in the 0.3% NaCl group. Treatment with the high dose of pravastatin resulted in a significant decrease in the amount of BNP mRNA. Furthermore, the amounts of collagen I, collagen III, and fibronectin mRNAs were greater in the 8% NaCl group than in the 0.3% NaCl group, and these increases were prevented by treatment with the high dose of pravastatin.

3.5. Myocyte hypertrophy and collagen deposition

Light microscopic analysis revealed that the high-salt diet increased the cross-sectional area of cardiac myocytes apparent at 18 weeks of age (Fig. 3A). Treatment with pravastatin tended to reduce the magnitude of this increase but the effect was not significant. The extents of interstitial (Fig. 3B) and perivascular (Fig. 3C) fibrosis were also significantly increased by the high-salt diet; treatment with the high dose of pravastatin significantly inhibited these effects.

3.6. GSH/GSSG ratio and NADPH oxidase activity

The high-salt diet reduced the GSH/GSSG ratio and increased the NADPH oxidase activity in the left ventricle of DS rats (Fig. 4A). Treatment with the high dose of pravastatin prevented both of these effects of the 8% NaCl diet. The high-salt diet increased the relative amounts of the NADPH oxidase components p47phox and p67phox in the total membrane fraction of the left ventricle (Fig. 4B), indicative of the translocation of these proteins to membranes. The high-salt diet also induced the translocation of

![Graph showing GSH/GSSG ratio and NADPH oxidase activity](image)

![Immunoblot analysis of p47phox and p67phox](image)

Fig. 4. GSH/GSSG ratio, NADPH oxidase activity, and membrane translocation of p47phox and p67phox in the left ventricle of DS rats at 18 weeks of age. (A) GSH/GSSG ratio and NADPH oxidase activity. Data are means±SEM of values from six rats per group. (B) Immunoblot analysis of the amounts of p47phox and p67phox in cytosolic and total membrane fractions of the left ventricle. Both representative blots and quantitation of the ratio of the amounts of each protein in the membrane (M) and cytosolic (C) fractions are shown. Quantitative data are expressed relative to the values for the 0.3% NaCl group and are means±SEM of values from four rats per group. *P<0.05 vs. rats fed the 0.3% NaCl diet; †P<0.05 vs. rats fed the 8% NaCl diet without pravastatin.
Fig. 5. Membrane translocation of small GTPases in the left ventricle of DS rats at 18 weeks of age. The amounts of Rac1, H-Ras, and RhoA in cytosolic and total membrane fractions of the left ventricle were determined by immunoblot analysis. Both representative blots and quantitation of the ratio of the amounts of each protein in the membrane (M) and cytosolic (C) fractions are shown. Quantitative data are expressed relative to the values for the 0.3% NaCl group and are means±SEM of values from four rats per group. *P<0.05 vs. rats fed the 0.3% NaCl diet; †P<0.05 vs. rats fed the 8% NaCl diet without pravastatin.

Fig. 6. Expression and activity of MMPs and TIMPs in the left ventricle of DS rats at 18 weeks of age. (A) The amounts of MMP2, MMP9, TIMP1, and TIMP2 mRNAs in the left ventricle of DS rats in the four experimental groups were determined by quantitative RT-PCR analysis and normalized by the abundance of GAPDH mRNA. Data are means±SEM of values from six rats per group. (B) The activities of MMP2 and MMP9 and the abundance of TIMP1 and TIMP2 in the left ventricle were also determined. Data are means±SEM of values from five or six rats per group. *P<0.05 vs. rats fed the 0.3% NaCl diet; †P<0.05 vs. rats fed the 8% NaCl diet without pravastatin.
the small GTPases Rac1, H-Ras, and RhoA to membranes (Fig. 5). All of these effects of the 8% NaCl diet on protein translocation were prevented by treatment with the high dose of pravastatin.

3.7. Gene expression and activity of MMPs and TIMPs

The abundance of MMP2, MMP9, TIMP1, and TIMP2 mRNAs in the left ventricle was significantly greater in the 8% NaCl group than in the 0.3% NaCl group at 18 weeks of age (Fig. 6A). Treatment with the high dose of pravastatin significantly reduced the amounts of these mRNAs. A similar pattern of changes was observed with the activities of MMP2 and MMP9 and with the abundance of TIMP1 and TIMP2 proteins in the left ventricle (Fig. 6B).

4. Discussion

We have shown that treatment with pravastatin markedly improved the survival rate of DS rats fed a high-salt diet. Pravastatin also largely prevented the increase in LVDd and the decreases in both early diastolic MV and LV fractional shortening induced by the high-salt diet, suggesting that the statin ameliorated both diastolic and systolic dysfunction in this rat model of heart failure.

Although LV hypertrophy is an important predictor of heart failure [26], it remains unclear how the development of LV hypertrophy is regulated and what facilitates the transition from compensatory hypertrophy to the decompensatory stage. In the present study, treatment of DS rats fed the high-salt diet with pravastatin improved indices of diastolic and systolic function as determined by echocardiography; prevented the increase in the abundance of MMP2 mRNA in the left ventricle, which is closely related to the symptoms and outcome of LV dysfunction [27]; and increased survival rate. Treatment with statins has previously been shown to prevent the development of cardiac hypertrophy and to ameliorate heart failure in rats [12]. Although the high dose of pravastatin did not attenuate LV hypertrophy at 12 or 18 weeks of age in the present study, the treatment ameliorated LV dysfunction. These results suggest that the effects of treatment were not attributable to an antihypertensive or antihypertrophic action of the drug but rather were due to beneficial influences on ventricular structure and function.

Recent experimental and clinical investigations have revealed that statins exert various cholesterol-independent cardioprotective effects [14–16]. Many of these pleiotropic effects reflect the ability of statins to inhibit the synthesis of isoprenoid intermediates [28], which play a role in intracellular signaling. In particular, inhibition of small GTPases such as members of the Ras and Rho families, whose proper membrane localization and function are dependent on isoprenylation, may be important in the biological effects of statins. The activation of NADPH oxidase is achieved either by posttranslational modification of regulatory subunits and their translocation together with Rac1, a member of the Rho family, to the membrane or by an increase in subunit expression [29]. We have now shown that pravastatin inhibited the translocation of Rac1 and the NADPH oxidase subunits p47phox and p67phox from a cytosolic to a membrane fraction of the left ventricle of DS rats fed the high-salt diet. In addition, the increase in the production of superoxide by NADPH oxidase in the left ventricle that was induced by the 8% NaCl diet was prevented by pravastatin treatment. Pravastatin thus appeared to reduce the production of ROS by inhibiting the translocation of Rac1 and NADPH oxidase subunits to the membrane of LV myocytes. These results suggest that increased oxidative stress contributes to cardiac damage associated with salt-sensitive hypertension, consistent with the previous observations that NADPH oxidase-dependent ROS production increased progressively during the development of compensated hypertrophy and peaked at the stage of decompensated heart failure [1,30]. In the present study, pravastatin also prevented the decrease in the GSH / GSSG ratio in the left ventricle of DS rats fed the high-salt diet, suggesting that it delayed the transition from compensated LV hypertrophy to heart failure by reducing oxidative stress.

NADPH oxidase is a major source of ROS in cardiovascular cells including vascular smooth muscle cells, endothelial cells, myocytes, and fibroblasts. The activation of NADPH oxidase is thought to stimulate both the accumulation of collagen and extracellular matrix deposition by modulating the expression of inflammatory cytokines and inducing collagen synthesis by fibroblasts [31]. Cardiac fibroblasts are mainly responsible for the synthesis of extracellular collagen I, collagen III, and fibronectin in the heart. In the present study, the amounts of collagen I, collagen III, and fibronectin mRNAs were significantly increased, together with NADPH oxidase activity, in the left ventricle of 18-week-old DS rats fed the high-salt diet. ROS also activate MMPs produced by macrophage-derived foam cells in atherosclerotic plaques [6]. Relative MMP2 and MMP9 levels have been found to be increased in the LV myocardium of humans with end-stage heart failure [7,8] and rats with hypertensive heart failure [32]. Consistent with these observations, we have now shown that the activities of MMP2 and MMP9 as well as the abundance of the corresponding mRNAs were significantly increased in a manner sensitive to pravastatin in the left ventricle of 18-week-old DS rats fed the high-salt diet. Our results suggest that the activities and expression of MMPs are up-regulated in response to the oxidative stress generated by the mechanical strain associated with ventricular dysfunction, and that the increased MMP activity contributes to the progression to heart failure.

MMP activity is strictly regulated by inhibitors, including the MMP-specific family of TIMPs. An imbalance between MMPs and TIMPs has been thought to underlie pathological remodeling of the heart. The expression of
TIMPs is decreased in the failing human heart [25] and TIMP1-deficient mice manifest an increase in LV end-diastolic volume [33], suggesting that a reduction in myocardial collagen content contributes to the increased LV dilation associated with cardiomyopathies. In the present study, the expression of TIMP1 and TIMP2 was significantly increased at both the mRNA and protein levels in the left ventricle of 18-week-old DS rats fed the high-salt diet. Other studies have also detected an increased expression of TIMP1 and TIMP2 in the LV myocardium of humans or rats with decompensated hypertrophy [7,32]. Such an increase in TIMP abundance might reflect a compensatory response to the up-regulation of MMPs. Transcription of TIMP genes is regulated by cytokines, such as interleukin-1, interleukin-6, and transforming growth factor-β [34], that are strongly associated with the transition to heart failure. The up-regulation of TIMPs in the failing heart might thus be induced directly by cytokines.

The activation of MMPs is mediated by proteinases such as plasmin, trypsin, chymase, elastase, and kallikrein, with plasmin being thought to be an especially important physiological activator [9]. Urokinase-like plasminogen activator (uPA), which generates plasmin from plasminogen, affects remodeling of the interstitial collagen matrix and contributes to the development of chronic heart failure [35]. We did not detect a significant difference in the activity of uPA in plasma or the left ventricle between 18-week-old DS rats fed the high-salt diet and those fed the low-salt diet (data not shown), suggesting that uPA was not responsible for the changes in MMP activity in this rat model of heart failure. Signaling by the Ras and extracellular signal-regulated kinase (ERK) pathway has been shown to mediate the increase in MMP production induced by various stimuli [36]. The translocation of H-Ras to the membrane fraction of the left ventricle of DS rats in response to the high-salt diet observed in the present study might thus contribute to the observed activation of MMPs in this animal model.

Several members of the statin family suppress the expression of MMPs in macrophages and endothelial cells [16,37]. Furthermore, the suppression of MMP expression by statins results in an increase in the stability of atherosclerotic plaques and a decreased thrombogenicity [38]. Fluvastatin both increased the survival rate in a murine model of postinfarction heart failure and attenuated the associated increase in MMP activity [39]. We have now shown that pravastatin significantly inhibited the increases in the activities of MMP2 and MMP9 in the left ventricle that accompany hypertensive heart failure. Treatment with an MMP inhibitor was also found to reduce the oxidative stress and contractile dysfunction associated with heart failure induced by volume overload [40]. Prevention of the transition from compensated hypertrophy to heart failure by pravastatin might thus be due in part to a reduction in oxidative stress caused by suppression of MMP activity. Treatment with statins has previously been shown to reduce the activity of PAI-1 [15]. However, in the present study, pravastatin did not attenuate the increase in the activity of PAI-1 in plasma or the left ventricle of 18-week-old DS rats fed the high-salt diet (data not shown).

There are several limitations to the present study. First, previous studies have shown that statins inhibit the development of LV hypertrophy in vivo and in vitro and that this action is due to prevention of the translocation of small GTPases to the membrane [11–13]. Although we also found that treatment with the high dose of pravastatin prevented such translocation of small GTPases, it did not suppress the development of compensated hypertensive LV hypertrophy. These results suggest that other signaling pathways, such as those mediated by phosphoinositide 3-kinase and Akt or by calcineurin and NFAT3, might play an important role in the development of hypertrophy in this model. Second, detection of a 20% difference (60% vs. 80%, for example) in survival between two groups of animals with a two-sided significance level of 0.05 and a power of 80% would require 82 animals per group. In the present study, to minimize the number of animals used, we selected only 12 or 24 rats per group to examine the effect of pravastatin on survival. Nevertheless, treatment with the high dose of pravastatin was found by standard Kaplan–Meier analysis to induce a significant increase in the survival rate of DS rats on the high-salt diet.

In conclusion, although treatment with pravastatin did not prevent the development of compensated hypertensive LV hypertrophy in DS rats fed a high-salt diet, it ameliorated the LV relaxation abnormality and systolic dysfunction and prevented the transition to heart failure. The beneficial effects of pravastatin were associated with inhibition of increases both in the activities of MMPs and in the production of superoxide induced in the left ventricle of these rats by a high-salt diet.

Acknowledgments

We thank Akira Yamada, Tomoko Kato, and Mitsunori Iwase for assistance with echocardiographic measurements, Aya Matsushita and Kazuko Matsuba for assistance with morphological analysis, Toyoaki Murohara for helpful comments, and Ai Mizutani for help in preparation of the manuscript.

References


Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. Hypertension 2002;41:477–84.


