Original Article

Inhibition of Cardiac Remodeling by Pravastatin Is Associated with Amelioration of Endoplasmic Reticulum Stress

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The aim of this study is to investigate whether pravastatin can inhibit cardiac remodeling and ameliorate endoplasmic reticulum (ER) stress caused by pressure overload or tumor necrosis factor α (TNFα). Either pravastatin (5 mg/kg/d) or vehicle alone was orally administered to male C57BL/6J mice from day 2 after a transverse aortic constriction (TAC) was performed. The ER stress signaling pathway was also studied in pressure-overloaded hearts and in cultured cardiomyocytes treated with TNFα. Four weeks after TAC, pravastatin treatment significantly reduced heart/body weight and lung/body weight ratios and increased left ventricular (LV) fractional shortening compared with the TAC alone. Markers of ER stress, such as increases in ER chaperone and C/EBP homologous protein (CHOP) expression and enhanced phosphorylation of anti-phospho-eukaryotic initiation factor 2α (eIF2α), were observed in the hearts of TAC mice, while pravastatin treatment significantly blunted these changes. Pravastatin-treated TAC mice also showed less cardiac apoptosis. Cardiac expression of TNFα was increased in TAC mice, and TNFα induced ER stress in cultured neonatal rat cardiomyocytes, either of which was significantly inhibited by pravastatin. These findings indicate that pravastatin inhibits cardiac remodeling in mice subjected to pressure overload, and that this action is associated with inhibition of the ER stress signaling pathway. (*Hypertens Res* 2008; 31: 1977–1987)

***Key Words*: statins, endoplasmic reticulum stress, heart failure, hypertrophy, tumor necrosis factor α**

Introduction

The endoplasmic reticulum (ER) is classically characterized as an organelle that participates in the folding of membrane and secretory proteins (*1*). Various cellular stresses, including ischemia, hypoxia, gene mutation, oxidative stress, and increased protein synthesis, lead to impairment of ER function, and stimuli that cause ER dysfunction are collectively termed ER stress (*1–4*). There is emerging evidence that ER stress plays an important role in cardiovascular diseases such as atherosclerosis (*3*, *5*) and chronic heart failure (*6*). A recent report from our laboratory revealed that prolonged ER stress contributes to the progression from cardiac hypertrophy to heart failure, and that the apoptosis pathway headed by the C/EBP homologous protein CHOP—a transcription factor induced by ER stress—is activated in failing hearts (*6*). These results suggest that inhibition of ER stress could be an alter-

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native therapeutic approach to treating heart failure.

There is a large body of evidence that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) may protect the heart *via* various effects independent of their lipid-lowering action (*7*). These effects include modulation of cell signaling pathways that influence the expression of inflammatory factors (*8*, *9*), an anti-oxidant effect (*10*), enhancement of the bioavailability of endothelial nitric oxide (*11–13*), inhibition of protein synthesis (*14*) and inhibition of sympathetic nervous activity (*15*). Since the production of inflammatory cytokines (*16*), oxidative reactions (*3*, *16*, *17*), protein synthesis (*2*), and sympathetic activation (*18*) are all reported to be closely associated with ER stress, it would seem logical to speculate that amelioration of ER stress might be another effect of statins. It was recently reported that tumor necrosis factor α (TNFα) could induce ER stress in murine fibrosarcoma cells (*16*) and in intestinal epithelial cells (*19*) and that TNFα knockout mice showed less cardiac remodeling in response to pressure overload (*20*). However, it is still unknown whether TNFα can induce ER stress in cardiomyocytes and whether statins can inhibit such putative TNFα-induced ER stress. We hypothesized that pressure overload might induce ER stress through upregulation of myocardial TNFα expression, subsequently leading to heart failure, while statin therapy might inhibit cardiac remodeling through the suppression of TNFα production and TNFα-induced ER stress. To test these hypotheses, we designed the present study to examine whether pravastatin has a beneficial effect on cardiac remodeling in mice subjected to pressure overload, and whether pravastatin could inhibit ER stress induced by either pressure overload or TNFα.

Methods

Transverse Aortic Constriction Model and Experimental Protocol

All procedures were performed in accordance with our institutional guidelines for animal research, which conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised in 1996). Male C57BL/6J mice (7–8 weeks old and weighing 20–24 g) were anesthetized with a mixture of intraperitoneal xylazine (5 mg/kg) and ketamine (100 mg/kg). Transverse aortic constriction (TAC) was performed to induce cardiac hypertrophy and heart failure, as described previously (*21*, *22*). The mortality rate of mice in this laboratory was about 30% for the murine TAC model. The degree of hypertrophy and heart failure increased in a time-dependent manner; usually, the wet heart/body weight ratio (HW/BW) increased by 80–90% at 4 weeks.

Mice were divided into three groups: the Sham group ($n=10$), the TAC group ($n=20$), and the TAC+pravastatin group ($n=20$). Pravastatin, an HMG-CoA reductase inhibitor (provided by Sankyo Pharmaceutical Co. Ltd., Tokyo, Japan), was administered daily at a dose of 5 mg/kg (dissolved in the drinking water) from day 2 after the TAC was performed. The dose of pravastatin was determined according to the results of our preliminary experiments, which showed that increasing the daily dose to 20 mg/kg did not further ameliorate heart failure. Echocardiography and left ventricle (LV) hemodynamic studies were done 4 weeks after the TAC procedure. Afterward the mice were sacrificed, and their hearts and lungs were extracted for further analysis. For histological examination, hearts were fixed in 10% formalin; those for Western blot analysis were snap-frozen in liquid nitrogen and stored at −80°C until use.

Echocardiography

Noninvasive transthoracic echocardiography was performed in conscious mice with a Sonos 4500 Echocardiography System and a 15-6 L MHz transducer (Philips, Eindhoven, the Netherlands). The animals were fixed in position without anesthesia. Two-dimensional short-axis views of the LV were obtained for guided M-mode measurements of the posterior wall thickness (LVPWd), end-diastolic diameter (LVEDd), and end-systolic diameter (LVESd). LV fractional shortening (LVFS) and the ejection fraction (LVEF) were calculated as follows:

$$\mathrm{LVFS} = (\mathrm{LVEDd} - \mathrm{LVESd})/\mathrm{LVEDd} \times 100,$$

$$\mathrm{LVEF} = [(\text{LV end-diastolic volume} - \text{LV systolic volume})/\text{LV end-diastolic volume}] \times 100.$$

The LV volume was calculated by the formula of Teichholz:

$$V = [7/(2.4 + D)] \times D^3,$$

where V is the LV volume and D is the LV dimension (*23*). LV mass was calculated according to a cubic assumption modified by the formula of Teichholz:

$$\text{LV mass (mg)} = 1.055[7(\mathrm{LVEDd} + \mathrm{LVPWd} + \mathrm{VSTd})^3/(2.4 + \mathrm{LVEDd} + \mathrm{LVPWd} + \mathrm{VSTd}) - \mathrm{LVEDV}],$$

where 1.055 is the gravity of the myocardium and VSTd is the diastolic ventricular septal thickness.

Invasive Hemodynamic Study

To determine the pressure gradient on the third day after the TAC procedure, three mice were randomly selected from each of the TAC and TAC+pravastatin groups and anesthetized as mentioned above, after which an endotracheal tube was inserted and connected to a volume-cycled rodent ventilator as described elsewhere (*22*). Ventilation was required to avoid respiratory arrest due to the ligation of both carotid arteries. A 1.4 F Millar pressure catheter (Millar Instruments, Houston, USA) was inserted into each of the left and right carotid arteries, and the pressures were measured simulta-

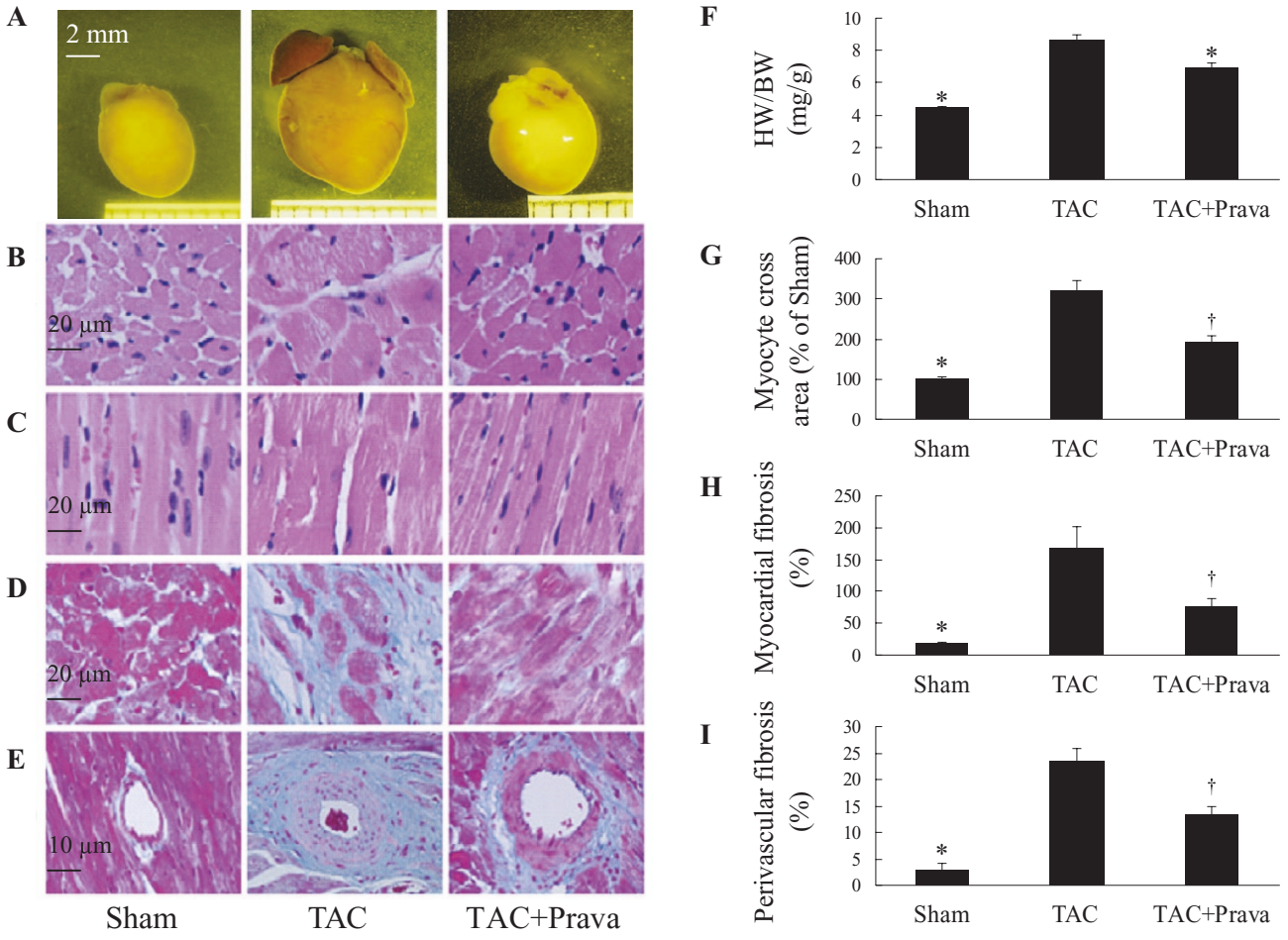

Fig. 1. *Effect of pravastatin (Prava) on cardiac hypertrophy and fibrosis in mice subjected to pressure overload. A: Representative pictures of the whole heart. B: Cross-sectional view of cardiomyocytes. C: Long-axis view of cardiomyocytes. D: Myocardial fibrosis. E: Perivascular fibrosis. The heart weight/body weight ratio (HW/BW) (F) and cardiomyocyte cross-sectional area (G) were significantly reduced in pravastatin-treated mice. Myocardial fibrosis (H) and perivascular fibrosis (I) were also inhibited by pravastatin. *$p < 0.01$, †$p < 0.05$, vs. TAC (transverse aortic constriction) by post-hoc analysis. In F, the number of mice is 9, 14, and 16 for the sham, TAC, and TAC+pravastatin groups, respectively. In G–I, 4 hearts from each group were used to obtain the data. Results are shown as the mean±SEM.*

neously by using a data acquisition and analysis system (PowerLab; AD Instruments, Nagoya, Japan). As a result, the systolic blood pressure gradient was found to be similar in the two groups. These mice were excluded from further assessment because they could not survive this procedure.

LV hemodynamics were evaluated at 4 weeks after the TAC procedure. Nine to eleven mice from each group were anesthetized (lightly for TAC mice) and were ventilated as mentioned above. A Millar catheter was inserted *via* the right carotid artery and carefully introduced into the LV to measure the LV systolic pressure (LVSP) and the LV end-diastolic pressure (LVEDP). The maximum and minimum rates of LV pressure change (max dp/dt and min dp/dt, respectively), as well as the contractility index (max dp/dt divided by the pressure at the time of max dp/dt) and the exponential time constant of relaxation (τ), were calculated using a software program (Blood Pressure Module; AD Instruments).

Histological Examinations

Hearts were fixed in 10% formalin, dehydrated, and then embedded in paraffin. Subsequently, 6 μm thick sections were cut and stained with hematoxylin eosin statin or Azan/Mallory statin. The cross-sectional areas of cardiomyocytes and of myocardial and perivascular fibrosis were quantified using four hearts from each group, as described previously (*24*, *25*). Briefly, myocardial fibrosis in the heart was quantitatively analyzed by morphometry. The perivascular fibrosis of arteries was evaluated from short-axis images of intramuscular arteries and arterioles in Azan/Mallory-stained sections.

Table 1. Echocardiographic Parameters at 4 Weeks Post-TAC

Parameter	Sham (n=10)	TAC (n=11)	TAC+Prava (n=13)	p value (ANOVA)
LVEDd (mm)	2.85±0.04*	3.22±0.06	2.92±0.11†	0.0191
LVPWd (mm)	0.65±0.01*	0.92±0.02	0.79±0.02*	<0.0001
LVESd (mm)	1.20±0.05*	2.1±0.01	1.53±0.09*	<0.0001
LVFS (%)	58.2±1.2*	34.8±1.4	48.0±1.9*	<0.0001
LVEF (%)	89±1*	65±3	80±2*	<0.0001
LV mass (mg)	48.4±1.4*	83.6±1.3	63.7±4.1*	<0.0001
HR (beats/min)	531.6±6.3	488.0±21.4	485.5±16.1	0.0514

TAC, transverse aortic constriction; LVEDd, left ventricular end-diastolic dimension; LVPWd, left ventricular diastolic posterior wall thickness; LVESd, left ventricular end-systolic dimension; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction; LV, left ventricular; HR, heart rate; TAC+Prava, TAC+pravastatin (5 mg/kg/d). *p<0.01 and †p<0.05 compared with TAC. Data in the first three columns are shown as the mean±SEM.

Table 2. Invasive Left Ventricular Hemodynamics at 4 Weeks Post-TAC

Parameter	Sham (n=9)	TAC (n=10)	TAC+Prava (n=10)	p value (ANOVA)
LVSP (mmHg)	87±2.6*	166±5.4	168±12.1	<0.0001
LVEDP (mmHg)	8.1±1.7*	24.4±1.6	16.5±1.4*	<0.0001
Max dp/dt (mmHg/s)	2,870±185	3,201±111	3,363±350	0.8945
Min dp/dt (mmHg/s)	2,560±129	2,995±177	3,540±413	0.2331
Contractility index	79.0±5.7*	44.6±2.0	46.2±3.9	<0.0001
τ (ms)	18.3±1.0*	25.4±1.3	23.8±1.6	0.0026

TAC, transverse aortic constriction; LVSP, maximum left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Max dp/dt, the steepest slope during the upstroke of the pressure curve; Min dp/dt, the steepest slope during the downstroke of the pressure curve; Contractility index, max dp/dt divided by the pressure at the time of max dp/dt; τ, the exponential time constant of relaxation; Prava, pravastatin at 5 mg/kg/d. *p<0.01 compared with TAC. Data in the first three columns are shown as the mean±SEM.

The area occupied by the artery (A) and the area of fibrosis surrounding the artery (B) were traced and calculated. The perivascular fibrosis index was defined as B/A. The entire area of each section was scanned at 200× magnification. Terminal dUTP nick-end labeling (TUNEL) and staining for TNFα (ENVISION system) were performed as described elsewhere (*26*). To determine the percentage of apoptotic cells, TUNEL-positive and -negative cells were both counted and expressed as follows:

$$\%\ \text{of apoptotic cells} = \text{the number of TUNEL-positive cells/total cells} \times 100.$$

Cell Culture

Ventricular myocytes were isolated from neonatal rats at 2 to 3 d of life and cultured as previously described (*27*). In brief, the cardiomyocytes were incubated for 72 h in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and then grown for 24 h under serum-free conditions. To examine whether pravastatin inhibits the production of TNFα, cardiomyocytes were exposed to lipopolysaccharide (LPS) (Sigma, St. Louis, USA) at a concentration of 0.1 μg/mL for 3, 6 or 24 h, with or without pretreatment of 10 μmol/L pravastatin at 2 h before LPS addition. Next, we investigated whether pravastatin inhibits TNFα-induced ER stress signals and whether these effects were mediated through HMG-CoA reductase–dependent pathways. Cells were pretreated with 10 μmol/L pravastatin for 2 h, and then exposed to 10 ng/mL TNFα (Sigma) for 24 h in the presence or absence of 5 μmol/L farnesyl pyrophosphate ammonium salt (FPP) (Sigma) or 5 μmol/L geranylgeranyl pyrophosphate ammonium salt (GGPP) (Sigma). ER stress signaling was examined by Western blotting.

Western Blot Analysis

Protein samples were prepared from whole-heart homogenates or cultured cardiomyocytes, as described elsewhere (*28*). Proteins (30–50 μg) were separated by 5% to 20% gradient denaturing SDS-PAGE. After transfer to membranes, immunoblot analysis was performed with the following antibodies: anti-GRP94 and anti-GRP78 (Stressgen Biotechnologies), anti-CHOP/GADD153 (Santa Cruz Biotechnology, Santa Cruz, USA), anti-phospho-eukaryotic initiation factor 2α (eIF2α) (Ser51) (Cell Signaling Technology, Danvers, USA), and anti-GAPDH (Chemicon International, Temecula, USA). Immunoreactive bands were visualized by the

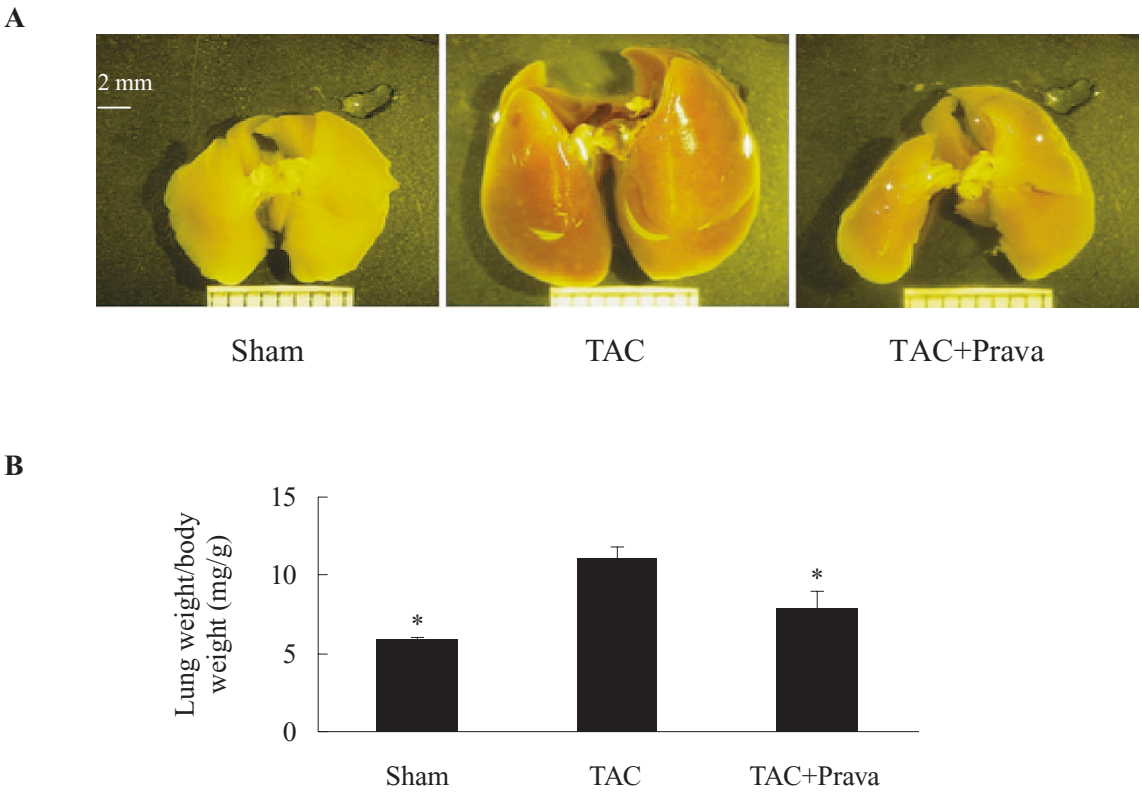

Fig. 2. *Pravastatin (Prava) reduces pulmonary congestion induced by pressure overload. A: Representative pictures of whole lungs show that pulmonary congestion was ameliorated by pravastatin treatment at 4 weeks after TAC. B: The lung weight/body weight ratio was significantly reduced by pravastatin treatment (the number of mice in each group is the same as in Fig. 1F), *$p < 0.01$. Data are shown as the mean±SEM.*

enhanced chemiluminescence method (Amersham, Buckinghamshire, UK), and then the band intensity was quantified using NIH Image software.

TNFα levels were measured by using an ELISA kit (Quantikine, Catalog No. MTA00; R&D SYSTEMS, Minneapolis, USA) according to the manufacturer's instructions. Either 250 μg protein or 50 μL cell culture supernatant was loaded in each well.

Statistical Analysis

The unpaired Student's *t*-test was used for comparisons between two groups, and one-way ANOVA with post hoc analysis by the Tukey-Kramer test was employed for multiple comparisons. Results are expressed as the mean±SEM and $p<0.05$ was considered to indicate statistical significance.

Results

Pravastatin Inhibits Cardiac Hypertrophy and Fibrosis

Four weeks after TAC, the wet HW/BW and cardiomyocyte cross-sectional area were markedly increased in the TAC group compared with the sham group. Daily administration of pravastatin at 5 mg/kg (TAC+pravastatin group) significantly inhibited cardiomyocyte hypertrophy (Fig. 1A–C, F and G). We also observed an inhibitory effect of pravastatin on myocardial and perivascular fibrosis (Fig. 1D, E, H and I).

Pravastatin Reduces Cardiac Remodeling and Improves Heart Failure

The mortality rate of mice was 30% (6 of 20 mice) in the TAC group and 20% (4 of 20 mice) in the TAC+pravastatin group. No significant difference was found between the two groups. Echocardiographic examination of conscious mice revealed the following differences between the groups. Compared with sham-operated mice, TAC mice showed a significant increase of LV wall thickness, LV dimension and LV mass, as well as a decrease in LVFS and LVEF. The TAC+pravastatin group had thinner LV walls, a smaller LV dimension and lower LV mass, as well as a larger LVFS and LVEF, than the TAC group. No significant differences in heart rate were found among the three groups (Table 1). These findings suggested that pravastatin improved LV systolic function and also inhib-

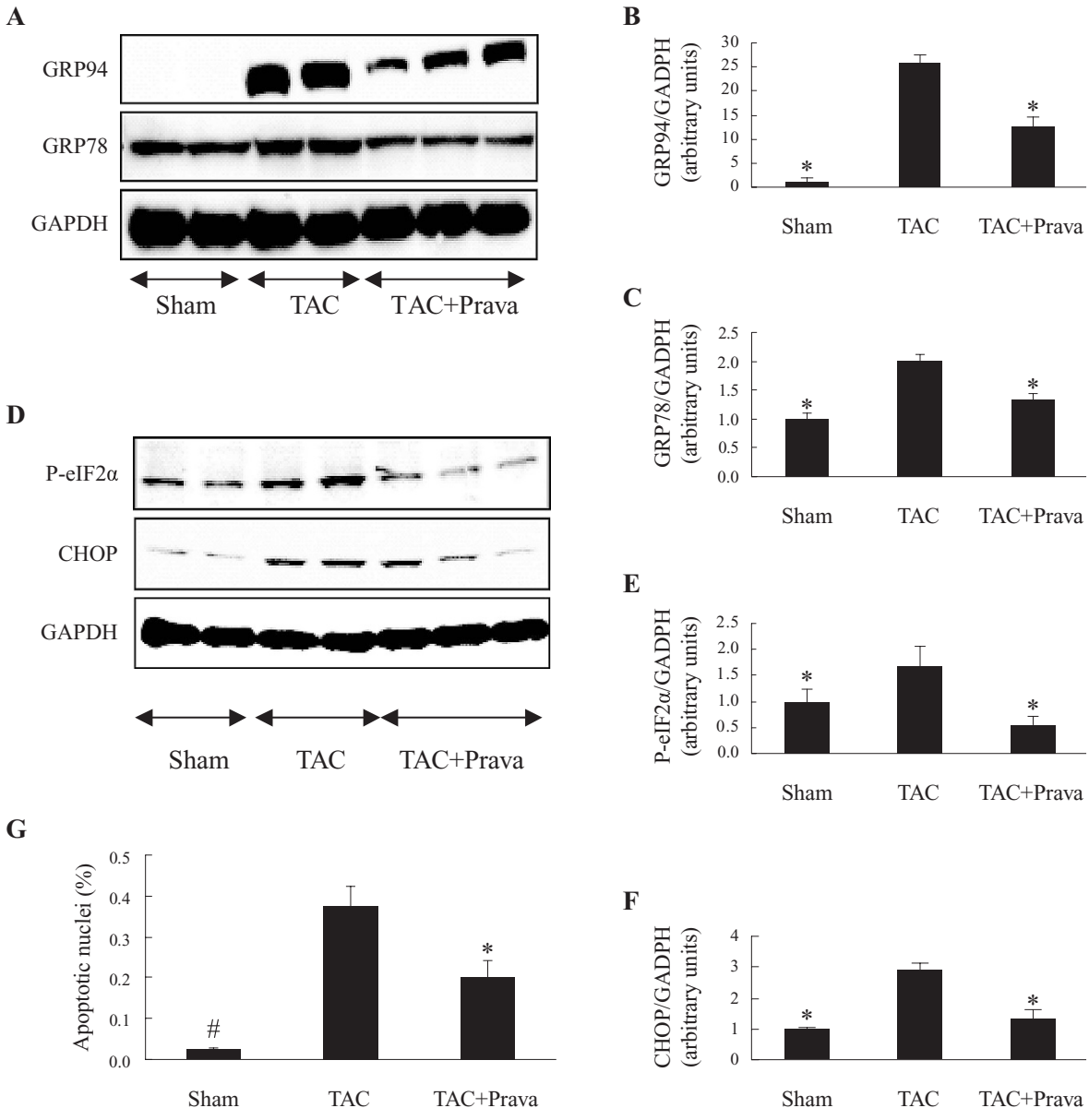

Fig. 3. *Pravastatin (Prava) decreases the expression of chaperone proteins and inhibits ER stress–induced apoptotic signals. A: Representative Western blot showing expression of GRP94 and GRP78. B: Quantitative expression of GRP94. C: Quantitative expression of GRP78. D: Representative Western blot showing expression of phosphorylated eukaryotic initiation factor 2α (P-eIF2α) and CHOP. E: Quantitative expression of P-eIF2α. F: Quantitative expression of CHOP. G: Pravastatin reduced the rate of cardiomyocyte apoptosis, since TUNEL-stained nuclei were increased in the TAC group and were markedly decreased in the pravastatin-treated TAC mice. Each group had 3–5 animals, *$p<0.05$, #$p<0.01$ vs. TAC. Data are shown as the mean±SEM.*

ited LV remodeling.

LV hemodynamics was evaluated by using a Millar pressure catheter before sacrificing the animals. As a result, LV pressure overload was found to be similar in the TAC mice with and without pravastatin treatment. Despite this result, LVEDP was significantly lower in the pravastatin-treated mice, and there were also improvements in max d*p*/d*t* and min d*p*/d*t*, contractility index, and τ, although the differences were not significant (Table 2).

Pulmonary congestion was also examined as an index of cardiac function in this model. It was found that exposure to pressure overload for 4 weeks caused a significant increase of the lung/body weight (LW/BW) ratio, and that this increase was inhibited by pravastatin treatment (Fig. 2).

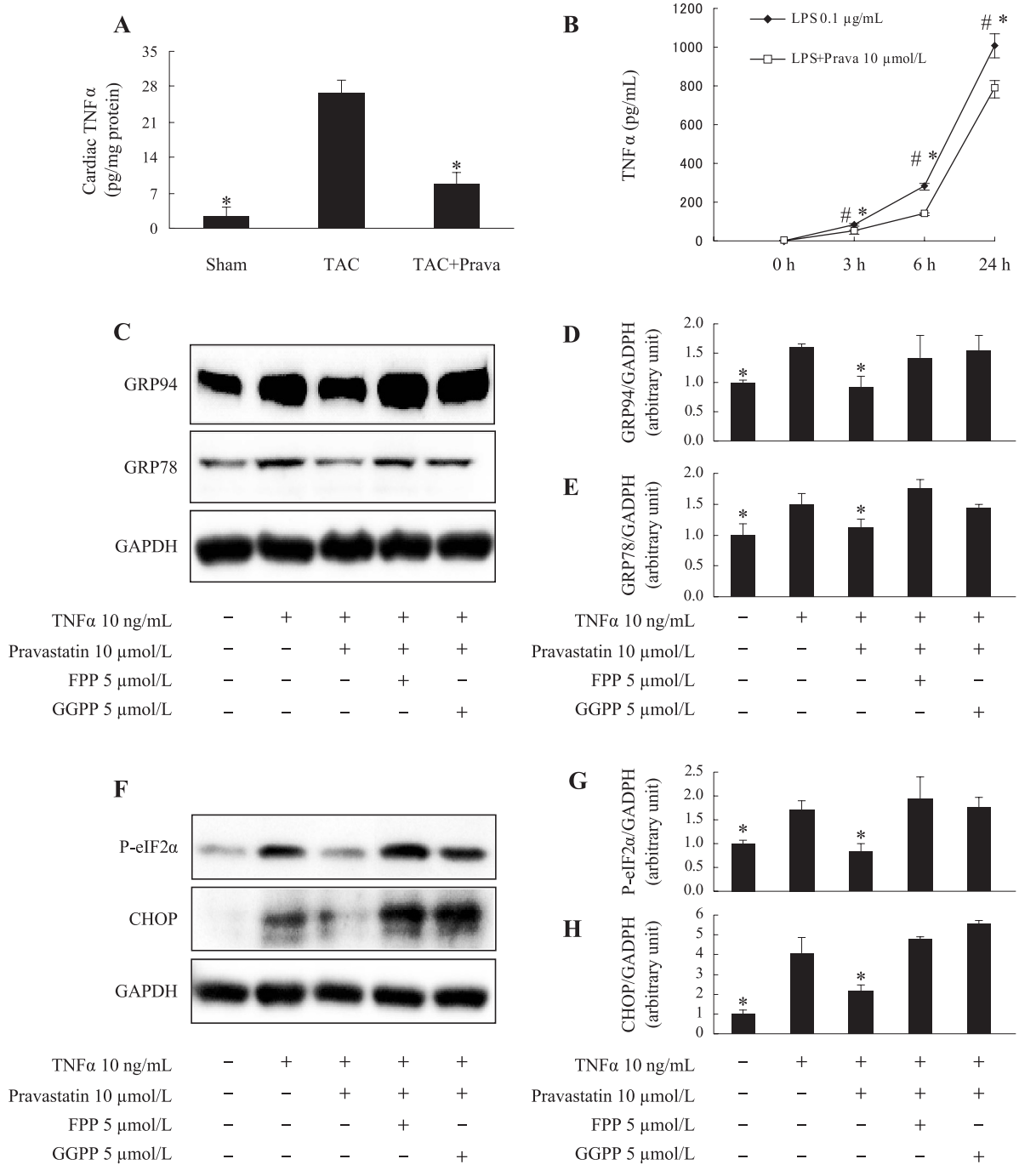

Fig. 4. *Pravastatin (Prava) inhibits TNFα induced ER stress in cultured neonatal rat cardiomyocytes A: Cardiac expression of TNFα detected by ELISA. B: Pravastatin inhibited the TNFα production induced by LPS. *$p < 0.01$ vs. 0 h; #$p < 0.01$ vs. LPS+Prava group. At the time point of 0 h, TNFα levels were measured before LPS addition in the LPS group and after 2 h of pretreatment with pravastatin without LPS in the LPS+Prava group, $n = 5$ for every time point for both groups. C: Pravastatin alleviated the increase of GRP78 and GRP94 induced by TNFα. Representative Western blot showing expression of GRP94 and GRP78. D: Quantitative expression of GRP94. E: Quantitative expression of GRP78. F: TNFα-induced phosphorylation of eIF2α and CHOP was inhibited by pravastatin. Representative Western blot showing expression of phosphorylated eukaryotic initiation factor 2α (P-eIF2α) and CHOP. G: Quantitative expression of P-eIF2α. H: Quantitative expression of CHOP. FPP, farnesyl pyrophosphate ammonium salt; GGPP, geranylgeranyl pyrophosphate ammonium salt. Each experiment was repeated at least three times. Data are shown as the mean±SEM. *$p < 0.05$ vs. TNFα in D, E, G, H.*

These findings indicated that pravastatin treatment could inhibit cardiac hypertrophy and improve heart failure. Next, we investigated whether these beneficial effects of pravastatin were associated with the inhibition of ER stress.

Pravastatin Decreases the Expression of ER Chaperones

We first examined the translational induction of two ER-specific chaperones (GRP78 and GRP94) involved in the ER stress/unfolding protein response. Consistent with our previous report (*6*), cardiac expression of ER chaperones was significantly upregulated by TAC, and treatment with pravastatin suppressed this pressure overload–induced increase of GRP78 and GRP94 (Fig. 3A–C).

Inhibition of ER-Initiated Apoptotic Signaling by Pravastatin

Since persistent ER stress can initiate the apoptotic process by the transcriptional induction of CHOP (*29*), we also examined the effect of pravastatin on this signaling pathway. As shown in Fig. 3D–F, expression of phosphorylated eIF2α and CHOP were both strongly induced in the hearts of TAC mice, and pravastatin treatment significantly attenuated the increase of these two proteins.

Pravastatin Decreases Myocyte Apoptosis

Since the apoptotic signals induced by ER stress eventually lead to apoptosis, we assessed cardiomyocyte apoptosis by TUNEL staining (percentage of positive nuclei) in each group. TUNEL staining showed very low levels of apoptosis in sham-operated mice, whereas it was increased markedly in TAC mice. In contrast, pravastatin-treated mice showed a significantly lower rate of cardiomyocyte apoptosis (Fig. 3G).

Cardiac TNFα Levels Are Increased in Heart Failure

Cardiac expression of TNFα (detected by ELISA) was elevated in TAC mice. Treatment with pravastatin significantly decreased TNFα expression but did not completely inhibit it (Fig. 4A). Besides reducing the cardiac production of TNFα, it would probably be of therapeutic value if pravastatin could prevent TNFα-induced cell damage. Therefore, we next attempted to examine whether pravastatin inhibited TNFα production in cardiomyocytes.

Pravastatin Inhibits LPS-Induced TNFα Production in Cardiomyocytes

In cultured neonatal rat cardiomyocytes, LPS (0.1 μg/mL) increased TNFα production in a time-dependent manner, and these responses were significantly inhibited by pravastatin (10 μmol/L) treatment (Fig. 4B). Since pravastatin could inhibit TNFα production by only 20–50%, we wondered whether the remaining high levels of TNFα would further detrimentally influence cardiomyocytes. Therefore we examined whether TNFα could induce ER stress and, if so, whether pravastatin had an inhibitory effect on such TNFα-induced ER stress in cardiomyocytes.

Pravastatin Inhibits TNFα-Induced ER Stress Signals

In cultured neonatal rat cardiomyocytes, treatment with TNFα for 24 h significantly induced ER stress, as evidenced by the increase of chaperone proteins and the activation of the unfolded protein response (UPR)– and ER stress–related apoptotic signal pathways. Co-treatment with pravastatin markedly decreased the expression of chaperone proteins GRP78 and GRP94 (Fig. 4C–E), and also downregulated the expression of phospho-eIF2α and CHOP (Fig. 4F–H). The co-administration of FPP or GGPP abolished the effects of pravastatin on TNFα-induced ER stress (Fig. 4C–H).

Discussion

The beneficial effects of pravastatin observed in this study included the amelioration of cardiomyocyte hypertrophy as well as improved pulmonary congestion, diminished cardiac fibrosis, increased left ventricular contractility (LVFS and LVEF), and decreased LVEDP. We also found that pravastatin treatment reduced the expression of chaperone proteins and inhibited ER stress–induced apoptotic signaling in hearts subjected to pressure overload. Moreover, we observed that pravastatin not only decreased TNFα production but also inhibited the induction of ER stress by this cytokine. We first confirmed that TNFα was able to induce ER stress in cardiomyocytes and that pravastatin inhibited ER stress due to either *in vivo* mechanical or *in vitro* stimulation with TNFα.

Statin therapy for patients with either ischemic or nonischemic heart failure has attracted a great deal of interest, but most of the studies reported to date have been retrospective. Our research group carried out a small prospective trial that demonstrated a beneficial effect of short-term statin therapy on dilated cardiomyopathy, with significant improvement of both echocardiographic LV function and New York Heart Association (NYHA) functional class compared with a placebo (*30*). Several other clinical studies have also shown that statin therapy is beneficial for heart failure (*31–33*), although negative reports have appeared as well (*34*). Noticeably, a recent report showed that rosuvastatin treatment reduced the total number of heart failure hospitalizations in a large-scale randomized double-blind placebo-controlled trial (*35*). Another large-scale trial that is still ongoing (*36*, *37*) may eventually clarify the impact of statin therapy on heart failure. Meanwhile, experimental studies are also helpful in assessing the effects of statins in the setting of nonischemic heart fail-

ure, as well as in exploring the underlying mechanisms. The results of the present study support the concept that statin therapy is beneficial for nonischemic heart failure. Most notably, our findings suggest that amelioration of ER stress might be a new effect of statins.

In a previous study performed in our laboratory (*6*), we demonstrated that the ER-dependent apoptotic pathway is one of the mechanisms involved in the progression from cardiac hypertrophy to heart failure. Cytokines (*16*, *38*), norepinephrine (*18*), and angiotensin II (*6*) have critical roles in the pathophysiology of heart failure, and all were reported to induce the UPR. Therefore, it may not be surprising that ER stress plays an important role in the pathophysiology of heart failure. Induction of ER chaperones is an early adoptive response to ER stress, and is also a mark of the severity of stress. In this study, we found that pravastatin downregulated two ER chaperone proteins (GRP94 and GRP78) that were induced by either pressure overload or TNFα. This result suggests two possibilities; one is that this drug ameliorates ER stress as an early effect that subsequently leads to reduced expression of ER chaperones; the other is that pravastatin reduces ER chaperone expression without improving ER stress. Because we found that pravastatin inhibited the apoptotic signaling induced by ER stress, it is reasonable to assume that the reduction of chaperone protein expression in pravastatin-treated mice results from ER stress inhibition.

If severe ER stress persists, apoptotic pathways are activated, including the induction of CHOP, caspase 12, and c-Jun NH_2-terminal kinase (JNK) (*29*). It has been reported that overexpression of CHOP leads to apoptosis (*39*), whereas deficiency of CHOP reduces apoptosis due to ER stress (*40*, *41*). Furthermore, our previous study showed that CHOP belonged to the main pathway involved in murine heart failure (*6*), suggesting that CHOP has a critical role in the process of cardiac cell death caused by ER stress. Therefore, we focused on the CHOP-dependent apoptosis pathway in the present study. We observed that induction of CHOP and apoptosis were both increased in failing hearts, and that their expression levels were significantly decreased by pravastatin therapy. Several previous studies also support our finding that statins inhibit apoptosis in diseased hearts (*39*, *40*). These findings suggest that pravastatin-attenuated cardiac remodeling is due to pressure overload along with the amelioration of CHOP-dependent apoptosis pathways.

Recent reports have provided new evidence that TNFα has a critical role in the occurrence of heart failure in mice (*20*, *41*). Since inflammatory cytokines are thought to have a role in heart failure and were reported to induce ER stress, we chose TNFα as a target. Consistent with previous studies, we observed the elevation of cardiac TNFα levels in TAC mice. In addition, we found that pravastatin reduced the cardiac expression of TNFα. Although we did not identify the mechanisms by which pravastatin can attenuate cardiac TNFα levels, we speculate that there are two possible explanations for that phenomenon. The first is the inhibition of TNFα expression by statins *via* modulation of NADPH oxidase activity and reactive oxygen species generation (*42*). The second is that statins modify TNFα production by inhibiting the activation of nuclear transcription factor, NF-κB (*43*, *44*). Further investigation will be needed to identify the mechanism.

Several reports have clearly demonstrated that pravastatin was effective in cultured cells, it suppressed the proliferation of cardiac fibroblasts (*45*), increased the expression of adiponectin receptor 1 that was reduced by TNFα (*46*), and inhibited H_2O_2- or hypoxia and reoxygeneration–induced apoptosis (*47–49*), all of which were in good agreement with our results. On the other hand, there is evidence that pravastatin did not affect protein synthesis induced by interleukin-1β (*50*), endothelin (*51*) or angiotensin II (*52*) or nitrite accumulation stimulated by interleukin-1β in cardiomyocytes (*53*). The precise mechanisms of these diverse effects of statins on cardiomyocytes need to be clarified in future studies. Our results showed that the inhibitory effect of pravastatin on TNFα-induced ER stress could be abolished by FPP or GGPP, metabolic products of cholesterol, indicating that pravastatin attenuates TNFα-induced ER stress signaling by inhibiting HMG-CoA reductase.

Although it has been reported that TNFα induces the unfolded protein response in cells other than cardiomyocytes (*16*), it has not been clear whether TNFα can induce ER stress in the heart. Our findings in this study provide evidence that TNFα induces ER stress in cardiomyocytes. Although we found that short-term treatment of TNFα did not induce apoptosis in cultured cardiomyocytes (data not shown), we cannot deny the possibility that prolonged ER stress due to TNFα may contribute to the progression of apoptosis in diseased hearts.

In conclusion, pravastatin effectively suppressed cardiac remodeling induced by pressure overload in mice, and this effect was associated with amelioration of ER stress induced by pressure overload or TNFα. Besides uncovering a new action of statins, these results could be clinically relevant for the treatment of heart failure.

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