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Attenuation of oxidative stress and cardiac dysfunction by bisoprolol in an animal model of dilated cardiomyopathy

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Abstract

Oxidative stress is an important susceptibility factor for dilated cardiomyopathy. We have investigated the effects of bisoprolol, a β_1-selective adrenoceptor blocker, on oxidative stress and the development of cardiac dysfunction in a model of dilated cardiomyopathy. Male TO-2 and control hamsters at 8 weeks of age were treated with bisoprolol (5 mg/kg per day) or vehicle for 4 weeks. Treatment with bisoprolol prevented the progression of cardiac dysfunction in TO-2 hamsters. This drug did not affect the increase in NADPH oxidase activity but prevented the reduction in activity and expression of mitochondrial manganese-dependent superoxide dismutase as well as the increases in the concentrations of interleukin-1β and tumor necrosis factor-α in the left ventricle of TO-2 hamsters. Attenuation of the development of cardiac dysfunction by bisoprolol may thus result in part from normalization of the associated increases in the levels of oxidative stress and pro-inflammatory cytokines in the left ventricle.

Keywords: Adrenoceptors; Cardiomyopathy; Cytokines; Heart failure; NADPH oxidase

Dilated cardiomyopathy (DCM) is a cardiac muscle disease characterized by progressive ventricular dilation and loss of cardiac function, and is the most common cause of severe heart failure and disability in younger adults [1]. Recent studies suggest that an increase in the level of oxidative stress resulting from increased cardiac generation of reactive oxygen species (ROS) contributes to contractile and endothelial dysfunction, myocyte apoptosis and necrosis, and remodeling of the extracellular matrix in the heart [2,3]. Superoxide production or biochemical markers of oxidative stress have thus been found to be increased in individuals with DCM [4]. Oxidative stress is therefore considered an important susceptibility factor for DCM, with agents that reduce the level of such stress or interfere with the generation of intracellular ROS having potential for the treatment of DCM patients.

Controlled clinical trials have shown that long-term administration of the β-blockers metoprolol, nebivolol, bucindolol, carvedilol, or bisoprolol increases ventricular function and improves clinical status in certain patients with DCM or severe chronic heart failure [5–9]. Experimental data have suggested that the beneficial effects of β-blockers on left ventricular (LV) function in heart failure might depend on a reduction in heart rate [10]. Certain β-blockers, such as carvedilol and metoprolol, have been

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shown to reduce the level of lipid peroxidation evident in the myocardium of DCM patients [11] and to inhibit the up-regulation of the DNA binding activities of redox-regulated transcription factors in neonatal rat cardiac ventricular myocytes [12], suggesting that one of the beneficial effects of β-blockers in individuals with heart failure is attenuation of oxidative stress. Bisoprolol exhibits a high selectivity for β_1-adrenoceptors and was found in the CIBIS II study to greatly reduce mortality in patients with ischemic or nonischemic heart failure [9]. Moreover, the efficacy of this agent was recently shown to be similar to that of angiotensin-converting enzyme inhibitors for initiation of treatment of chronic heart failure in the CIBIS III study [13]. However, it has remained unknown whether bisoprolol reduces the level of oxidative stress in the failing LV myocardium. With the use of TO-2 cardiomyopathic hamsters, we have now investigated the effects of bisoprolol on the progression of cardiac dysfunction as well as on lipid peroxidation and protein nitration, and the levels of pro-inflammatory cytokines in the LV myocardium.

Materials and methods

Experimental animals. Male cardiomyopathic Syrian hamsters (BIO TO-2) and male control hamsters (BIO F1B) were obtained at 5 weeks of age from BIO Breeders (Fitchburg, MA). TO-2 hamsters, which have a deletion of the δ-sarcoglycan gene, have been extensively studied as a model of DCM [14] and are an appropriate model with which to characterize the role of myocardial oxidative stress from the onset of LV dysfunction to overt heart failure. The animals were maintained under constant environmental conditions, with a 12-h-light, 12-h-dark cycle (light on from 08.00 to 20.00 h) and with free access to food and water. All experimental procedures were performed in accordance with Institutional Guidelines for Animal Research, and the study was approved by the Animal Ethics Committee of Nagoya University Graduate School of Medicine.

Study protocol. At 8 weeks of age, F1B and TO-2 hamsters were each randomly assigned to one of two groups: those treated with vehicle (F1B group, $n = 6$; TO-2 group, $n = 8$) and those treated with bisoprolol at a dose of 5 mg per kilogram of body weight per day (F1B + Biso group, $n = 6$; TO-2 + Biso group, $n = 8$). Bisoprolol (Tanabe, Osaka, Japan) or vehicle (saline) was administered orally by gastric gavage once a day for 4 weeks. At the completion of treatment, the heart was excised immediately after physiological measurements.

Assessment of LV function and physiological measurements. Transthoracic echocardiography with a 13-MHz transducer (Acuson Sequoia 512) was performed on hamsters anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) at 6 weeks of age (before random assignment to treatment groups), 9 weeks of age (1 week after treatment initiation), and 12 weeks of age (at completion of treatment), as previously described [15]. The thickness of the interventricular septum (IVST) and the 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. Systolic blood pressure and heart rate were measured noninvasively at the left brachial artery by a modified tail-cuff method (MK-2000; Muromachi Kikai, Tokyo, Japan), as previously described [16], after the animals had been anesthetized with sodium pentobarbital as described for echocardiography.

Assay of glutathione and NADPH oxidase activity. The left ventricle was separated from the atria and the right ventricle, weighed, and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. The amount of total glutathione [reduced (GSH) plus oxidized (GSSG)] in the left ventricle was determined as described [17] with a recycling assay based on glutathione reductase and 5,5′-dithiobis-(2-nitrobenzoic acid). The amount of GSSG was determined by Griffith's method [18] after the addition of 2-vinylpyridine to the assay mixture. 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 [19]. 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. Lucigenin chemiluminescence was expressed as counts per minute per milligram of protein.

Measurement of 4-hydroxynonenal (4-HNE). The concentration of 4-HNE was measured as described [20]. In brief, 250 μl of 0.05 M *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride (Fluka, Buchs, Switzerland) were mixed with 50 μl LV homogenate. 4-Hydroxybenzaldehyde (Merck, Hohenbrunn, Germany) was used as an internal standard. The mixture was incubated at room temperature for 30 min, after which 0.5 ml of methanol, 2 ml of hexane, and six drops of concentrated sulfuric acid were added. The hexane fraction was separated by centrifugation, dried under nitrogen, and subjected to derivatization for 2 h at 80 °C with 40 μl *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane (Supelco, Bellefonte, PA). The resulting sample was then analyzed by gas chromatography and mass spectrometry with negative-ion chemical ionization. Acquisition of mass spectra was performed in the ion-monitoring mode selected for mass/charge (m/z) ratios of 403 and 369 for 4-HNE and 4-hydroxybenzaldehyde derivatives, respectively.

Assay of glutathione peroxidase (GSHPx), catalase, and superoxide dismutase (SOD) activities. GSHPx activity was determined as previously described [21], with hydrogen peroxide as the substrate and the rate of disappearance of NADPH at 37 °C recorded spectrophotometrically at 340 nm. Assay of catalase activity was based on reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde thereby produced was measured spectrophotometrically at 540 nm as previously described [22]. Total SOD activity in cytosolic and mitochondrial fractions of LV homogenates was assayed spectrophotometrically at 405 nm as described [23] and was expressed in units per milligram of protein. One unit of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. After the addition of potassium cyanide to the assay mixture, manganese-dependent SOD (MnSOD) activity was determined as previously described [24].

Immunoblot analysis. Cytosolic and mitochondrial fractions were isolated from LV tissue and subjected to immunoblot analysis with rabbit polyclonal antibodies to copper- and zinc-dependent SOD (Cu/ZnSOD) (1:4000 dilution) or to MnSOD (1:5000 dilution) (Abcam, Cambridge, UK). Immune complexes were detected with enhanced chemiluminescence (ECL) reagents (GE Healthcare Bio-Science, Piscataway, NJ). Band intensities were quantified with the use of Quantity One Image software (Bio-Rad, Hercules, CA).

Histology and immunohistochemistry. Midventricular slices was rapidly isolated, frozen, and equatorially sectioned at a thickness of 5 μm. The frozen sections were fixed with ice-cold 4% paraformaldehyde for 10 min and stained with Azan Mallory solution for evaluation of the extent of fibrosis. Alternatively, the fixed sections were incubated for 20 min at room temperature with 10% normal goat serum in phosphate-buffered saline and then subjected to immunostaining with rabbit polyclonal antibody to 3-nitrotyrosine (1:80 dilution; Upstate Biotechnology, Lake Placid, NY) as previously described [25].

ELISAs for interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Frozen LV tissue was homogenized on ice and assayed for IL-1β (Pierce/Endogen, Rockford, IL) and TNF-α (Bioscience, San Diego, CA) with enzyme-linked immunosorbent assay (ELISA) kits as described [26]. Assays were performed in duplicate and absorbance at 450 nm was measured with a microtiter plate reader. The tissue content of IL-1β or TNF-α is expressed as picograms per milligram of protein.

Statistical analysis. Data are presented as means ± SEM. Paired data were analyzed by the paired Student's t test. Differences among four groups were evaluated by one-way analysis of variance followed by

Dunnett's post hoc test. A P value of <0.05 was considered statistically significant.

Results

Body weight, ventricle weights, systolic blood pressure, and heart rate

Body weight was greater in the F1B group than in the TO-2 group and was not affected by treatment with bisoprolol (5 mg/kg per day) in either strain of hamster (Table 1). The weight of the left or right ventricle was also greater in the F1B group than in the TO-2 group after treatment with vehicle for 4 weeks, but it was not affected by treatment with bisoprolol in either strain (Table 1). Systolic blood pressure did not differ between the F1B group and the TO-2 group at any age examined and was not affected by treatment with bisoprolol (Table 1); we performed preliminary experiments to select a dose of bisoprolol for the present study that did not affect blood pressure. Heart rate also did not differ between the F1B group and the TO-2 group at any age. Treatment with bisoprolol for 4 weeks resulted in a significant decrease in heart rate in both F1B and TO-2 hamsters, but there was no significant difference in heart rate between treated animals of the two strains (Table 1).

Echocardiographic findings

The IVST was significantly smaller in the TO-2 group than in the F1B group at all ages examined (Table 2). Treatment with bisoprolol, however, resulted in a significant increase in the IVST in TO-2 hamsters without affecting that in F1B hamsters. Whereas the LVDd did not differ between F1B and TO-2 groups at 6 weeks of age, it was significantly greater in the TO-2 group than in the F1B group at 9 and 12 weeks (Table 2). Treatment with bisoprolol resulted in a significant decrease in the LVDd after 1 or 4 weeks in TO-2 hamsters without an effect on that in F1B hamsters. LV fractional shortening also did not differ between F1B and TO-2 groups at 6 weeks of age, and the decrease in this index of contractility apparent in TO-2 hamsters at 9 and 12 weeks was reversed by treatment with bisoprolol (Table 2).

GSH/GSSG ratio, 4-HNE concentration, and NADPH oxidase activity in the left ventricle

The GSH/GSSG ratio in LV tissue at 12 weeks of age was smaller in the TO-2 group than in the F1B group. Treatment with bisoprolol prevented the decrease in the GSH/GSSG ratio in TO-2 hamsters without affecting this parameter in F1B hamsters (Fig. 1A). The concentration of 4-HNE (Fig. 1B) and the activity of NADPH oxidase (Fig. 1C) in the left ventricle were each significantly greater in the TO-2 group than in the F1B group at 12 weeks of age. Treatment with bisoprolol prevented the increase in 4-HNE abundance in TO-2 hamsters, whereas NADPH oxidase activity did not differ significantly between the TO-2 group and the TO-2 + Biso group.

Activities of antioxidant enzymes in the left ventricle

There was no significant difference in total SOD activity in the cytosolic fraction of the left ventricle among the four experimental groups at 12 weeks of age (Fig. 2A). Total SOD activity in the mitochondrial fraction of the left ventricle was significantly decreased in the TO-2 group compared with the F1B group at 12 weeks of age, whereas that in the TO-2 + Biso group did not differ significantly from that in the TO-2 group or the F1B group (Fig. 2B). MnSOD activities in both cytosolic (Fig. 2C) and mitochondrial (Fig. 2D) fractions were significantly smaller in the TO-2 group than in the F1B group. Whereas treatment with bisoprolol significantly inhibited the reduction in mitochondrial MnSOD activity in TO-2 hamsters, there was no significant difference in cytosolic MnSOD activity between the TO-2 + Biso group and either the TO-2 group or the F1B group. The activities of GSHPx (Fig. 2E) and

Table 1
Body weight, ventricle weights, systolic blood pressure, and heart rate in the four experimental groups before (6 weeks of age) as well as 1 week (9 weeks of age) and 4 weeks (12 weeks of age) after the onset of treatment

Parameter	Age (weeks)	F1B	F1B + Biso	TO-2	TO-2 + Biso
Body weight (g)	6	84.04 ± 3.10	88.80 ± 1.26	67.78 ± 1.55*	66.27 ± 0.78*
	9	104.16 ± 3.35	107.41 ± 2.24	84.52 ± 0.50*	84.86 ± 1.23*
	12	112.22 ± 3.11	107.41 ± 2.24	84.52 ± 0.50*	84.86 ± 1.23*
LV weight (mg)	12	235.7 ± 6.1	233.4 ± 7.6	192.3 ± 7.5*	181.8 ± 4.5*
RV weight (mg)	12	54.6 ± 3.3	54.7 ± 1.5	38.3 ± 1.0*	40.4 ± 1.2*
Systolic BP (mmHg)	6	91.04 ± 7.79	93.67 ± 3.85	95.21 ± 7.26	90.14 ± 4.13
	9	96.20 ± 6.07	89.83 ± 6.96	89.62 ± 5.45	93.05 ± 3.99
	12	94.42 ± 10.36	87.57 ± 5.23	93.83 ± 9.56	77.14 ± 4.43
Heart rate (bpm)	6	419.2 ± 7.1	416.3 ± 18.5	384.6 ± 6.8	405.1 ± 12.2
	9	386.4 ± 7.6	368.5 ± 10.3	372.6 ± 14.9	378.3 ± 7.5
	12	383.4 ± 18.3	331.8 ± 10.9*	365.2 ± 25.4	298.1 ± 8.6**

RV, right ventricular; BP, blood pressure. Data are means ± SEM of values from six or eight animals per group.
* $P < 0.05$ vs. age-matched F1B group.
** $P < 0.05$ vs. age-matched TO-2 group.

Table 2
Echocardiographic findings in the four experimental groups before (6 weeks of age) as well as 1 week (9 weeks of age) and 4 weeks (12 weeks of age) after the onset of treatment

Parameter	Age (weeks)	F1B	F1B + Biso	TO-2	TO-2 + Biso
IVST (mm)	6	1.08 ± 0.02	1.03 ± 0.03	0.84 ± 0.02*	0.84 ± 0.02*
	9	1.12 ± 0.02	1.12 ± 0.03	0.80 ± 0.03*	1.03 ± 0.04**
	12	1.16 ± 0.02	1.17 ± 0.02	0.78 ± 0.04*	0.90 ± 0.02**
LVDd (mm)	6	3.86 ± 0.07	3.92 ± 0.03	3.82 ± 0.05	3.83 ± 0.10
	9	4.10 ± 0.09	4.10 ± 0.12	4.56 ± 0.20*	3.94 ± 0.12**
	12	4.10 ± 0.19	4.20 ± 0.10	5.26 ± 0.14*	4.64 ± 0.10**
FS (%)	6	52.86 ± 0.77	55.73 ± 1.08	50.34 ± 1.75	51.60 ± 1.74
	9	51.30 ± 1.32	51.37 ± 1.37	38.00 ± 1.79*	46.24 ± 1.68**
	12	48.84 ± 0.77	49.18 ± 0.71	25.26 ± 1.68*	39.34 ± 1.51**

FS, fractional shortening. Data are means ± SEM of values from six or eight animals per group.
* $P < 0.05$ vs. age-matched F1B group.
** $P < 0.05$ vs. age-matched TO-2 group.

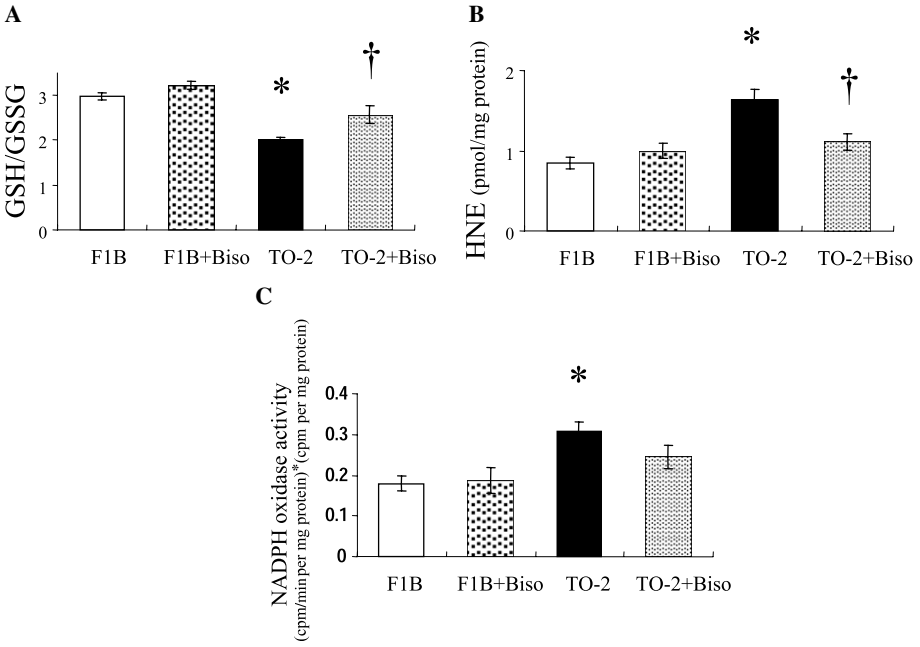

Fig. 1. Biological markers of oxidative stress in the left ventricle of animals in the four experimental groups after treatment for 4 weeks. The GSH/GSSG ratio (A), the concentration of 4-HNE (B), and NADPH oxidase activity (C) were determined in homogenates of LV tissue. Data are means ± SEM of values from six or eight hamsters per group. *$P < 0.05$ vs. F1B group; †$P < 0.05$ vs. TO-2 group.

catalase (Fig. 2F) did not differ among the four experimental groups at 12 weeks of age.

Abundance of SOD isoforms in the left ventricle

Immunoblot analysis revealed that there was no significant difference in the abundance of Cu/ZnSOD in the cytosolic fraction of LV homogenates among the four experimental groups at 12 weeks of age (Fig. 3A and B). The abundance of MnSOD in the mitochondrial fraction was significantly reduced in the TO-2 group compared with that in the F1B group, and treatment with bisoprolol resulted in a significant increase in the amount of mitochondrial MnSOD in TO-2 hamsters (Fig. 3A and C).

Fibrosis and 3-nitrotyrosine abundance in the left ventricle

The extent of interstitial fibrosis in the left ventricle at 12 weeks of age was markedly increased in the TO-2 group compared with that in the F1B group (Fig. 4A). This increase in cardiac fibrosis was greatly reduced by treatment of TO-2 hamsters with bisoprolol. Immunohistochemical staining of LV sections from animals at 12 weeks of age revealed a marked increase in the abundance of 3-nitrotyrosine in the TO-2 group compared with the F1B group (Fig. 4B). Treatment with bisoprolol greatly reduced the extent of this increase in the amount of 3-nitrotyrosine in TO-2 hamsters.

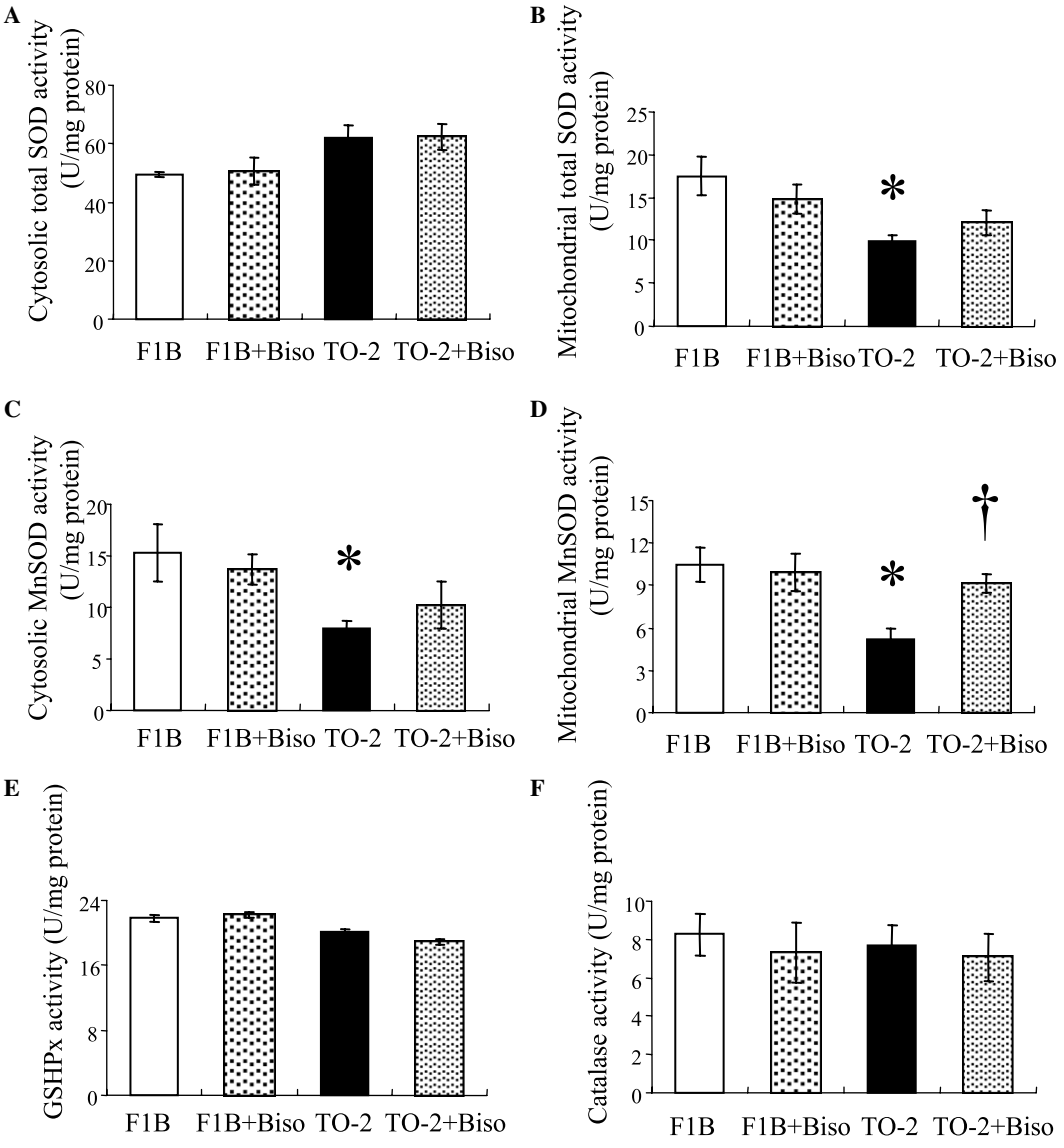

Fig. 2. Activities of SOD, GSHPx, and catalase in the left ventricle of animals in the four experimental groups after treatment for 4 weeks. Total SOD activity in cytosolic (A) and mitochondrial (B) fractions, MnSOD activity in cytosolic (C) and mitochondrial (D) fractions, and activities of GSHPx (E) and catalase (F) in homogenates of LV tissue were measured. Data are means ± SEM of values from six or eight hamsters per group. $^{*}P < 0.05$ vs. F1B group; $^{\dagger}P < 0.05$ vs. TO-2 group.

Concentrations of IL-1β and TNF-α in the left ventricle

The concentrations of both IL-1β and TNF-α in the left ventricle were significantly greater in the TO-2 group than in the F1B group at 12 weeks of age (Fig. 5). Treatment with bisoprolol prevented the increases in the tissue levels of IL-1β and TNF-α in TO-2 hamsters without affecting the amounts of these cytokines in F1B hamsters.

Discussion

We have shown that treatment with bisoprolol prevented the increase in LVDd and the decrease in LV fractional shortening in an animal of DCM. Bisoprolol also significantly inhibited the increases in the levels of oxidative stress and pro-inflammatory cytokines in the left ventricle that accompany the development of DCM in TO-2 hamsters.

An increase in the level of oxidative stress is implicated in the pathogenesis of heart failure, and inflammation is thought to play an important role in the progression of cardiovascular diseases [27]. Treatments that reduce the levels of oxidative stress or inflammation have thus been found to improve hemodynamic function in patients with advanced heart failure as well as in animal models of this condition [28,29]. DCM is a multifactorial disease, resulting from myocarditis, ischemic injury, or mitochondrial or genetic abnormalities. An increased generation of ROS is also an important susceptibility factor for DCM [3]. We recently showed that an increase in myocardial oxidative stress

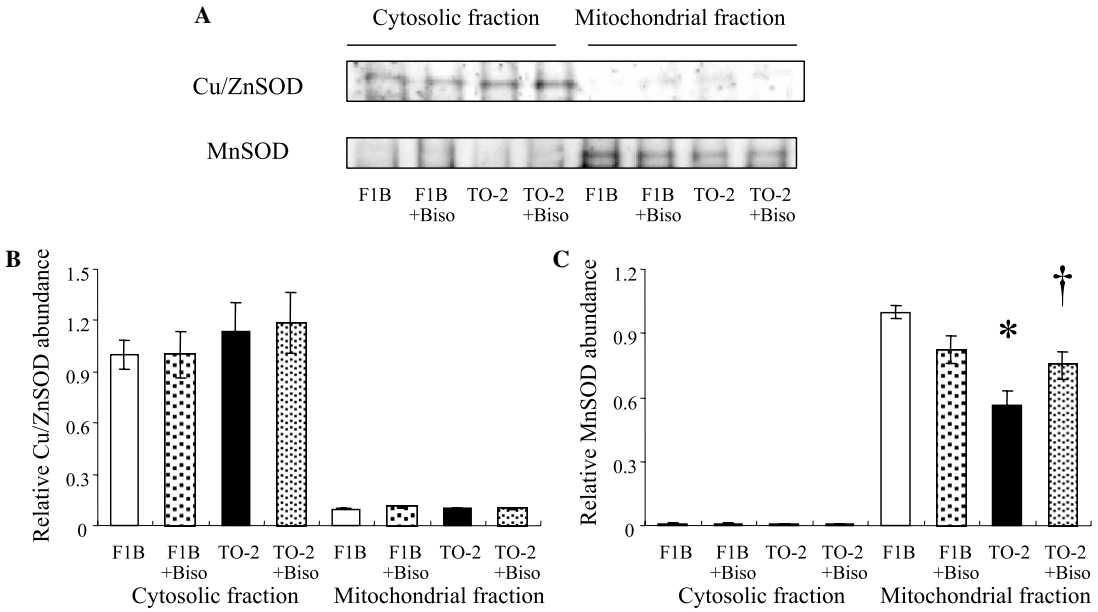

Fig. 3. Immunoblot analysis of Cu/ZnSOD and MnSOD in the left ventricle of animals in the four experimental groups after treatment for 4 weeks. (A) Representative blots of cytosolic and mitochondrial fractions. (B,C) Quantitation of the abundance of Cu/ZnSOD (B) and MnSOD (C) expressed relative to the corresponding value for the cytosolic of mitochondrial fraction, respectively, of the F1B group. Data are means ± SEM of values from five hamsters per group. $^{*}P < 0.05$ vs. F1B group; $^{\dagger}P < 0.05$ vs. TO-2 group.

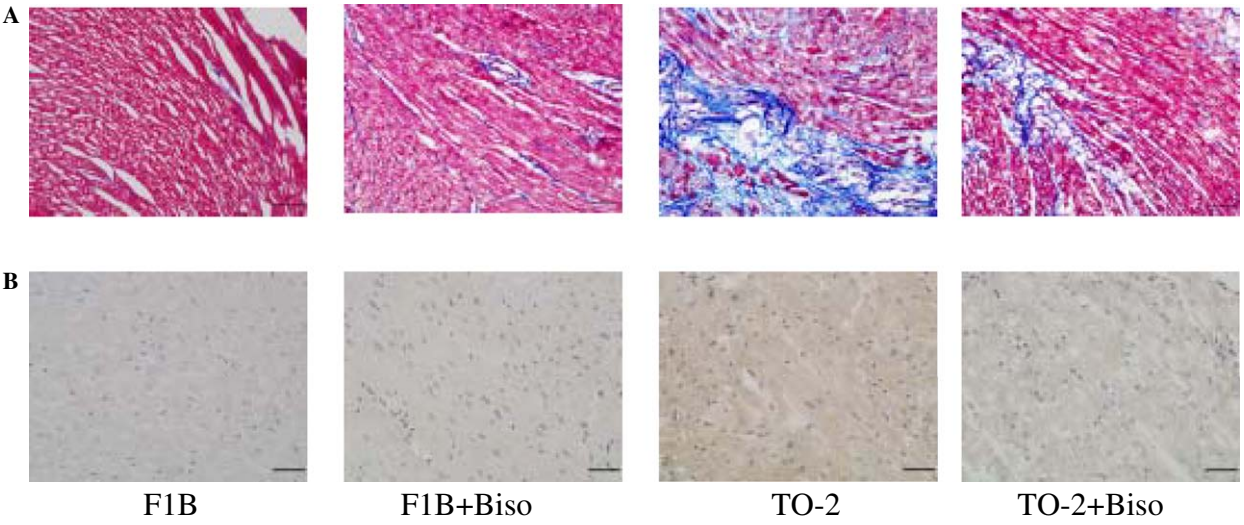

Fig. 4. Cardiac fibrosis and 3-nitrotyrosine accumulation in the left ventricle of animals in the four experimental groups after treatment for 4 weeks. (A) Interstitial collagen deposition as revealed by Azan Mallory staining. Bar, 100 μm. (B) Immunohistochemical staining for 3-nitrotyrosine. Bar, 50 μm.

(as reflected by a decrease in the GSH/GSSG ratio) is associated with impaired β-adrenergic signaling in TO-2 hamsters [30]. In the present study, we found that the concentration of 4-HNE, a biological marker of radical-induced lipid peroxidation during postischemic reperfusion injury of the myocardium [31], and the accumulation of 3-nitrotyrosine in the left ventricle were increased in TO-2 hamsters. This latter observation is consistent with previous results showing that the extent of protein nitration is correlated with that of cardiac dysfunction in vivo [32]. We also showed that the activity and abundance of MnSOD in the mitochondrial fraction were greatly decreased in the failing left ventricle of TO-2 hamsters, consistent with previous observations that MnSOD activity and abundance are markedly decreased in the failing heart of patients with DCM [33]. The increased level of oxidative stress that accompanies the initial development of LV dysfunction in TO-2 hamsters may thus contribute to cardiac damage in this model of DCM.

We found that bisoprolol suppressed the increases in the concentrations of both 4-HNE and 3-nitrotyrosine in the left ventricle of TO-2 hamsters. Bisoprolol thus appeared

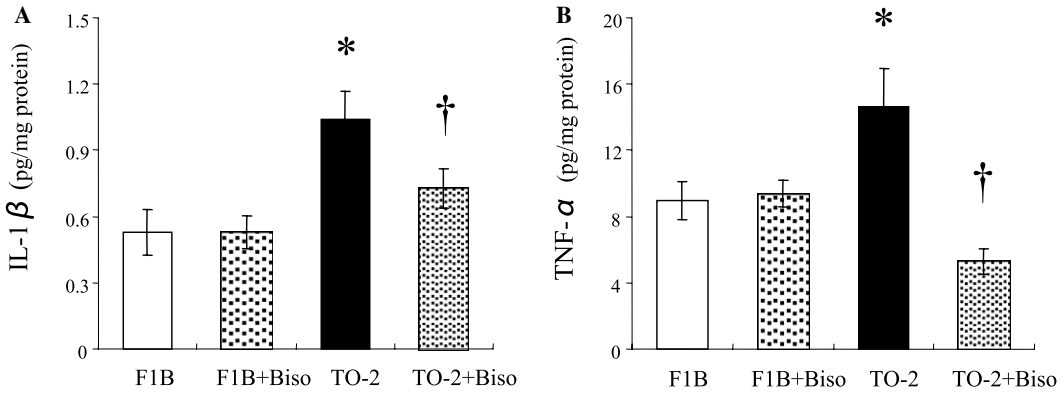

Fig. 5. Concentrations of pro-inflammatory cytokines in the left ventricle of animals in the four experimental groups after treatment for 4 weeks. The concentrations of IL-1β (A) and TNF-α (B) in homogenates of LV tissue were determined by ELISA. Data are means ± SEM of values from six or eight hamsters per group. $^{*}P < 0.05$ vs. F1B group; $^{\dagger}P < 0.05$ vs. TO-2 group.

to reduce the levels of lipid peroxidation and protein nitration mediated by biological oxidants in the myocardium of these animals. These results support the previous observation that another β-blocker, carvedilol, reduced the abundance of 4-HNE-modified proteins in the failing human myocardium [34]. Treatment of congestive heart failure due to systolic dysfunction with β-blockers can improve symptoms, increase the ejection fraction, and, probably, improve longevity [5–9]. Mechanisms proposed for the effectiveness of β-blockers in heart failure have included (i) improved β-adrenoreceptor function [35], (ii) protection of the myocardium from the effects of prolonged exposure to high levels of circulating catecholamines [36], and (iii) improvement of myocardial energetics as a result of the bradycardia that usually accompanies β-blockade [10]. In the present study, treatment with bisoprolol significantly reduced heart rate in both F1B and TO-2 hamsters. It also inhibited both the decrease in the GSH/GSSG ratio and the increases in lipid peroxidation and protein nitration in the left ventricle of TO-2 hamsters. Furthermore, bisoprolol attenuated the down-regulation of mitochondrial MnSOD in the left ventricle of these animals. These results suggest that normalization of the increased level of oxidative stress in the left ventricle that accompanies the development of cardiac dysfunction may contribute to the clinical efficacy of bisoprolol in heart failure. Among β-blockers tested, nebivolol and carvedilol, but not atenolol or metoprolol, were shown to prevent both uncoupling of vascular nitric oxide synthase 3 and activation of NADPH oxidase in inflammatory cells in animals with hyperlipidemia [37]. In the present study, bisoprolol prevented the increases in lipid peroxidation and protein nitration but did not inhibit the increase in NADPH oxidase activity in the left ventricle of TO-2 hamsters, suggesting that the mechanisms by which bisoprolol reduces the level of oxidative stress may differ, at least in part, from those that underlie this action of other β-blockers.

Pro-inflammatory cytokines, such as IL-1β and TNF-α, are induced by oxidative stress [38] and contribute to the development and progression of heart failure [4,39]. The concentrations of IL-1α, IL-1β, and TNF-α in plasma have been shown to be increased in patients with end-stage heart failure [40], and the expression of IL-1 in the myocardium was found to be increased in individuals with DCM [41]. Consistent with these previous observations, we have now shown that the concentrations of IL-1β and TNF-α in the left ventricle were increased in TO-2 hamsters. Pro-inflammatory cytokines produced by macrophages as well as by endothelial and myocardial cells contribute to myocyte apoptosis or hypertrophy, remodeling of the extracellular matrix, and loss of myocardial contractile function [42]. Several β-blockers have been shown to attenuate the expression of pro-inflammatory cytokines, including that of IL-1β and TNF-α, in the myocardium [43,44]. Our results are consistent with the notion that the normalization of the concentrations of IL-1β and TNF-α in the left ventricle of TO-2 hamsters may contribute to the prevention of the development of LV dysfunction by bisoprolol.

In the present study, we found that treatment with bisoprolol suppressed the increases in the levels of oxidative stress and pro-inflammatory cytokines in the left ventricle of TO-2 hamsters in parallel with prevention of the increase in LVDd and the decrease in LV fractional shortening. Whereas bisoprolol may directly affect myocardial oxidative stress, one limitation of our study is that we were not able to show whether the decreases in oxidative stress and cytokine abundance induced by this drug are the cause or result of the amelioration of DCM.

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