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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