Beneficial Effects of Growth Hormone-Releasing Peptide on Myocardial Oxidative Stress and Left Ventricular Dysfunction in Dilated Cardiomyopathic Hamsters

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Background: Growth hormone-releasing peptide (GHRP) may act directly on the myocardium and improve left ventricular (LV) function, suggesting a potential new approach to the treatment of cardiomyopathic hearts. The present study was hypothesis that the beneficial cardiac effects of GHRP might include attenuation of myocardial oxidative stress.

Methods and Results: Dilated cardiomyopathic TO-2 hamsters were injected with GHRP-2 (1 mg/kg) or saline from 6 to 12 weeks of age. F1B hamsters served as controls. Untreated TO-2 hamsters progressively developed LV dilation, wall thinning, and systolic dysfunction between 6 and 12 weeks of age. Marked myocardial fibrosis was apparent in untreated hamsters at 12 weeks of age in comparison with F1B controls. The ratio of reduced to oxidized glutathione (GSH/GSSG) was decreased and the concentration of 4-hydroxynonenal (4-HNE) was increased in the hearts of untreated TO-2 hamsters. Treatment with GHRP-2 attenuated the progression of LV remodeling and dysfunction, as well as myocardial fibrosis, in TO-2 hamsters. GHRP-2 also inhibited both the decrease in the GSH/GSSG ratio and the increase in the concentration of 4-HNE in the hearts of TO-2 hamsters.

Conclusions: GHRP-2 can suppress the increase in the level of myocardial oxidative stress, leading to attenuation of progressive LV remodeling and dysfunction in dilated cardiomyopathic hamsters. (Circ J 2010; 74: 163–170)

Key Words: Dilated cardiomyopathy; Growth hormone-releasing peptide; Hamsters; Heart failure; Oxidative stress

Dilated cardiomyopathy (DCM) is one of the most common causes of chronic heart failure and is a multifactorial disease, resulting from myocarditis, ischemia-induced injury, or mitochondrial or genetic abnormalities.1 Previous studies suggest that an increase in the level of oxidative stress resulting from increased cardiac generation of reactive oxygen species (ROS) might contribute to the contractile and endothelial dysfunction, myocyte apoptosis and necrosis, and remodeling of the extracellular matrix in the heart.2,3 Furthermore, superoxide production or biochemical marker of oxidative stress has 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.

The available treatments for DCM are palliative, and the prognosis of affected individuals remains poor. Fazio et al treated DCM patients with recombinant human growth hormone (GH) and observed improvement in left ventricular (LV) function, exercise capacity, and clinical status.5 On the other hand, Osterziel et al failed to detect any improvement in DCM patients treated with recombinant human GH in a randomized, double-blind, placebo-controlled study.6 An alternative approach to increasing the systemic levels of GH involves administration of a GH-releasing peptide (GHRP) or peptidomimetic agonist that stimulates GH secretion through direct action on the pituitary gland. Specific GHRP
binding sites are also present in the mammalian heart.\(^7\) The GHRP family includes ghrelin, hexarelin, GHRP-1, GHRP-2, and GHRP-6. We have previously reported that treatment with GHRP-6 can improve LV systolic performance and attenuate LV dilation during the progressive development of LV dysfunction in a TO-2 hamster model of DCM.\(^8\) Although it has been suggested that administration of GHRPs is a potential approach to the treatment of heart failure, the mechanism of the cardioprotective effects of GHRPs remains poorly understood. With the use of TO-2 cardiomyopathic hamsters, we therefore investigated the effects of GHRP-2 on myocardial oxidative stress and the progressive LV dysfunction in the present study. The TO-2 hamster with a mutation of the \(\delta\)-sarcoglycan gene has been extensively studied as a model of DCM.\(^9\) In addition, some cases of DCM in humans are also reported to result from \(\delta\)-sarcoglycan deficiency.\(^10\) The TO-2 hamster is characterized by progressive LV dilation, LV wall thinning, and LV systolic dysfunction.\(^11,12\) Recently, we found that myocardial oxidative stress was enhanced in the initial development of LV dysfunction in TO-2 hamsters.\(^12,14\) Therefore, TO-2 hamsters are an appropriate model with which to characterize the role of myocardial oxidative stress from the onset of LV dysfunction to overt heart failure.

**Methods**

**Experimental Animals and Study Protocol**

Male cardiomyopathic Syrian hamsters (TO-2 hamster, \(n=24\)) and male control hamsters (F1B hamster, \(n=8\)) were obtained at 5 weeks of age from BIO Breeders (Fitchburg, MA, USA). All animals were maintained under constant environmental conditions, with a 12-h-light, 12-h-dark cycle (light on from 08.00 to 20.00h) and with free access to food and water. Animal care was in accordance with institutional guidelines, and the experimental protocol was approved by the Committee on Laboratory Animal Utilization of Nagoya University. The investigation conformed to 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). Each day for 6 weeks TO-2 hamsters (6 weeks of age) were injected subcutaneously with low dose of GHRP-2 (0.01 mg/kg of body weight per day, \(n=8\)), high dose of GHRP-2 (1 mg/kg of body weight per day, \(n=8\)) (Kaken Pharmaceutical Co Ltd, Kyoto, Japan) or saline (\(n=8\)). Animals were weighed weekly.

**Echocardiography and Blood Pressure (BP) Measurement**

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) and at 12 weeks of age (completion of treatment). LV end-systolic (LVDs) and end-diastolic (LVDd) dimensions, interventricular septum thickness (IVST), and LV posterior wall thickness (LVPWT) were measured. LV fractional shortening (LVFS) was calculated as:

\[
\text{LVFS} = \frac{(\text{LVDd} - \text{LVDs})}{\text{LVDd}} \times 100.
\]

Mean LV wall thickness (LVWT) was defined as the average of the IVST and LVPWT. The ratio of LVDd and mean LVWT (LVDd/mean LVWT) was also calculated for the assessment of LV remodeling.\(^8,11\) To assess the response to \(\beta\)-adrenergic stimulation, we measured LV function after intraperitoneal injection of isoproterenol (5 \(\mu\)g/kg) at 12 weeks of age.\(^12\) Systolic BP and the heart rate (HR) were measured noninvasively at the left brachial artery using a modification of the tail-cuff method (MK-2000, Muromachi Kikai, Tokyo, Japan), as previously described,\(^13\) after the animals had been anesthetized with sodium pentobarbital as described for echocardiography.

**Histology**

After death, hearts were excised, rinsed with saline, and blotted dry. The atria were removed, and both the right ventricular free wall and LV free wall plus IVS were separated and weighed. These specimens were fixed by immersion in 20% phosphate-buffered formalin, embedded in paraffin, and sectioned at 4-\(\mu\)m thickness. The LV sections were stained with Azan-Mallory solution for evaluation of the extent of fibrosis.
GHRP in Hamsters With DCM

Assays of Myocardial Oxidative Stress and Antioxidants

LV homogenates were used for these assays. The amount of total glutathione [reduced (GSH) and oxidized (GSSG)] in the myocardium was determined by the glutathione reductase and 5,5’-dithiobis-(2-nitrobenzoic acid) recycling assay as previously described. The amount of GSSG was determined by Griffith’s method. The concentration of 4-hydroxynonenal (4-HNE) was measured by modification of a gas chromatography–mass spectrometry method as previously described.

Total superoxide dismutase (SOD) activity in cytosolic fraction was assayed by the spectroscopic method as previously described, and expressed as units of SOD/mg protein: 1 unit of SOD activity is defined as the amount of enzyme activity that causes 50% inhibition of nitroblue tetrazolium reduction. Glutathione peroxidase (GSHPx) activity was determined as previously described, using hydrogen peroxide as the substrate, and the rate of disappearance of NADPH was recorded spectrophotometrically (340nm) at 37°C.

Statistical Analysis

Values are expressed as mean±SEM. Multiple comparisons were analyzed by one-way analysis of variance followed by Fisher’s test. A P value <0.05 was considered statistically significant.

Results

Body Weight, LV Weight, Systolic BP, and HR

At 6 weeks of age, the body weight of the F1B control hamsters was significantly greater than that of the TO-2 hamsters (Figure 1). At 12 weeks of age, the body weight gain by TO-2 hamsters treated with high dose of GHRP-2 (+35.2±1.0g) appeared to be greater than that of either TO-2 hamsters with low dose of GHRP-2 (+31.5±1.4g) or untreated TO-2 hamsters (+31.0±1.7g); at this time, the body weight of TO-2 hamsters with a high dose of GHRP-2 was similar to that of F1B hamsters (Figure 1). LV weight, the ratio of LV weight to body weight, and systolic BP were significantly smaller in the TO-2 groups compared with F1B.

Table 1. BW, LVW, SBP, and HR in Hamsters at 12 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>F1B controls</th>
<th>TO-2 untreated</th>
<th>TO-2 with low GHRP-2</th>
<th>TO-2 with high GHRP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>98.5±1.8</td>
<td>92.3±2.5</td>
<td>92.8±2.4</td>
<td>97.2±2.6</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>222±6</td>
<td>193±5*</td>
<td>194±4*</td>
<td>201±7*</td>
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<tr>
<td>LVW/BW (mg/g)</td>
<td>2.26±0.05*</td>
<td>2.10±0.05*</td>
<td>2.10±0.04*</td>
<td>2.07±0.04*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>92±7</td>
<td>78±6*</td>
<td>71±3*</td>
<td>72±2*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>336±15</td>
<td>306±20</td>
<td>300±19</td>
<td>312±20</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *P<0.05 vs F1B hamsters.

BW, body weight; LVW, left ventricular weight; SBP, systolic blood pressure; HR, heart rate; GHRP, growth hormone-releasing peptide.

Table 2. Echocardiographic Data for Hamsters at 6 and 12 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>F1B controls</th>
<th>TO-2 untreated</th>
<th>TO-2 with low GHRP-2</th>
<th>TO-2 with high GHRP-2</th>
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<tr>
<td>LVDd (mm)</td>
<td></td>
<td></td>
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<tr>
<td>6 weeks</td>
<td>3.74±0.08</td>
<td>3.65±0.04</td>
<td>3.61±0.06</td>
<td>3.70±0.08</td>
</tr>
<tr>
<td>12 weeks</td>
<td>4.09±0.08</td>
<td>5.03±0.08*</td>
<td>4.73±0.12*</td>
<td>4.61±0.13*</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>6 weeks</td>
<td>1.75±0.07</td>
<td>1.71±0.04</td>
<td>1.63±0.06</td>
<td>1.84±0.07</td>
</tr>
<tr>
<td>12 weeks</td>
<td>2.08±0.08</td>
<td>3.75±0.10*</td>
<td>2.81±0.14*</td>
<td>2.71±0.12*</td>
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<tr>
<td>IVST (mm)</td>
<td></td>
<td></td>
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<tr>
<td>6 weeks</td>
<td>1.15±0.02</td>
<td>1.04±0.03*</td>
<td>1.05±0.03*</td>
<td>1.09±0.03</td>
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<tr>
<td>12 weeks</td>
<td>1.13±0.03</td>
<td>0.79±0.02*</td>
<td>0.93±0.03*</td>
<td>0.96±0.03*</td>
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<tr>
<td>LVPWT (mm)</td>
<td></td>
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<tr>
<td>6 weeks</td>
<td>1.15±0.03</td>
<td>0.98±0.03*</td>
<td>1.04±0.02*</td>
<td>1.10±0.03</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1.10±0.02</td>
<td>0.75±0.03*</td>
<td>0.93±0.03*</td>
<td>0.98±0.04*</td>
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<tr>
<td>LVDD/mean LVWT</td>
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<td></td>
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<td></td>
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<tr>
<td>6 weeks</td>
<td>3.26±0.08</td>
<td>3.64±0.08*</td>
<td>3.46±0.06</td>
<td>3.40±0.10</td>
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<td>12 weeks</td>
<td>3.69±0.13</td>
<td>6.57±0.21*</td>
<td>5.15±0.25*</td>
<td>4.80±0.21*</td>
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<tr>
<td>LVFS (%)</td>
<td></td>
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<tr>
<td>6 weeks</td>
<td>53.3±1.0</td>
<td>53.0±1.3</td>
<td>55.1±1.3</td>
<td>50.4±1.1</td>
</tr>
<tr>
<td>12 weeks</td>
<td>50.5±1.0</td>
<td>25.1±1.0*</td>
<td>40.6±1.6*</td>
<td>41.5±1.4*</td>
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<tr>
<td>ΔLVFS (%)</td>
<td></td>
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<tr>
<td>12 weeks</td>
<td>30.1±1.7</td>
<td>9.9±1.5*</td>
<td>15.7±2.2*</td>
<td>13.3±2.0*</td>
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</tbody>
</table>

Values are mean±SEM. *P<0.05 vs F1B hamsters, †P<0.05 vs untreated TO-2 hamsters.

GHRP, growth hormone-releasing peptide; LV, left ventricular; LVDd, LV end-diastolic dimension; LVDs, LV end-systolic dimension; IVST, interventricular septum thickness; LVPWT, LV posterior wall thickness; mean LVWT, mean LV wall thickness [(IVST+LVPWT)/2]; LVFS, LV fractional shortening; ΔLVFS, isoproterenol-induced increase in LVFS.
hamsters, but they were not affected by treatment with a low or high dose of GHRP-2 in TO-2 hamsters at 12 weeks of age (Table 1). HR was similar among all 4 groups at 12 weeks of age (Table 1).

**Echocardiographic Findings**

Echocardiographic findings are summarized in Table 2. At 6 weeks of age, LVDd and LVFS did not differ among all 4 groups. IVST tended to be smaller and the LVDd/mean LVWT ratio tended to be greater in TO-2 hamsters than in F1B hamsters, but they were similar among the 3 groups of TO-2 hamsters. At 12 weeks of age, LVDd and the LVDd/mean LVWT ratio were significantly greater in untreated TO-2 hamsters than in F1B hamsters. Treatment with either a low or a high dose of GHRP-2 attenuated the increases in LVDd and the LVDd/mean LVWT ratio in TO-2 hamsters. IVST and LVFS were significantly decreased in untreated TO-2 hamsters compared with F1B hamsters. Treatment with either a low or high dose of GHRP-2 ameliorated the decreases in IVST and LVFS in TO-2 hamsters. In addition, the response to β-adrenergic stimulation, as evaluated by the isoproterenol-induced increase in LVFS, was significantly decreased in untreated TO-2 hamsters compared with F1B hamsters at 12 weeks of age. Treatment with either a low or high dose of GHRP-2 increased the response to β-adrenergic stimulation in TO-2 hamsters at 12 weeks of age.
Myocardial Fibrosis
The extent of myocardial interstitial fibrosis was markedly increased in untreated TO-2 hamsters compared with F1B hamsters at 12 weeks of age. The increase in cardiac fibrosis was significantly reduced in TO-2 hamsters treated with a high dose of GHRP-2, but not a low dose (Figure 2).

Myocardial Oxidative Stress and Antioxidants
The GSH/GSSG ratio in the LV tissue was significantly smaller in untreated TO-2 hamsters than in F1B hamsters at 12 weeks of age. The decrease in the GSH/GSSG ratio was attenuated in TO-2 hamsters treated with a high dose of GHRP-2, but not a low dose (Figure 3A). The concentration of 4-HNE was significantly greater in untreated TO-2 hamsters compared with F1B hamsters at 12 weeks of age. Treatment with either a low or a high dose of GHRP-2 prevented the increase in the concentration of 4-HNE in TO-2 hamsters (Figure 3B). The activity of total SOD appeared to be smaller in untreated TO-2 hamsters than in F1B hamsters at 12 weeks of age, but there was no statistical difference. Treatment with either a low or a high dose of GHRP-2 significantly elevated the activity of total SOD in TO-2 hamsters compared with untreated TO-2 hamsters (Figure 3C). On the other hand, the activity of GSHPx did not differ among all 4 groups at 12 weeks of age (Figure 3D).

Discussion
A novel finding of the present study is that treatment with GHRP-2 suppressed the increase in the level of myocardial oxidative stress and ameliorated progressive LV remodeling and dysfunction in TO-2 cardiomyopathic hamsters. This suggests that administration of GHRP-2 is a potential new approach to the treatment of DCM and heart failure. To our knowledge, this is the first report of the beneficial effects of GHRP-2 on myocardial oxidative stress and cardiac dysfunction in DCM hamsters.

Effects of GHRP-2 on Systemic and Myocardial Growth
GHRPs, a family of small synthetic peptides, and their nonpeptide derivatives induce the release of GH both in vitro and in vivo through direct action on the pituitary gland. GH is potentially diabetogenic and may promote neoplastic growth. We have previously reported that treatment with GHRP-6 at a dose that had no effects on systemic or myocardial growth improved progressive LV remodeling and...
Effects of GHRP-2 on Myocardial Oxidative Stress and Antioxidants

Oxidative stress is reported to play a pivotal role in the pathogenesis and progression of heart failure,22,23 so inhibition of oxidative stress could be a potentially effective treatment.24–26 Our recent studies have shown that myocardial oxidative stress is enhanced in the initial development of LV dysfunction in TO-2 hamsters.15–17 In the present study, we demonstrated that the GSH/GSSG ratio was decreased in the failing heart of untreated TO-2 hamsters compared with F1B control hamsters at 12 weeks of age. We also found that the decrease in the GSH/GSSG ratio was attenuated in TO-2 hamsters treated with a high dose of GHRP-2, but not a low dose. 4-HNE has been proposed as an important marker of radical-induced lipid peroxidation during posts ischemic reperfusion injury of the myocardium.27 Nakamura et al demonstrated that myocardial levels of 4-HNE-modified protein in biopsy samples from patients with DCM were significantly increased compared with the levels in control subjects, suggesting that 4-HNE may play a critical role in the pathogenesis of heart failure.28 In the present study, treatment with either a low or a high dose of GHRP-2 prevented the increase in the concentration of 4-HNE in TO-2 hamsters at 12 weeks of age. GHRP-2 thus appeared to reduce the level of lipid peroxidation mediated by biological oxidants in the myocardium of TO-2 hamsters. We also found that treatment with GHRP-2 ameliorated progressive LV remodeling and dysfunction in TO-2 hamsters. The beneficial cardiac effects of GHRP-2 may be attributed to suppression of myocardial oxidative stress.

Oxidative stress occurs when the production of ROS exceeds the capacity of the antioxidant defense system. Primary antioxidant enzymes, including SOD, catalase, and peroxidase, work in parallel with nonenzymatic antioxidants to protect cells and tissues from ROS. A previous report demonstrated that homozygous knockout mice deficient in SOD die from DCM soon after birth.29 In the present study, total SOD activity appeared to be smaller in untreated TO-2 hamsters than in F1B hamsters at 12 weeks of age, but there was no statistical difference. In addition, treatment with either a low or a high dose of GHRP-2 significantly elevated the activity of total SOD in TO-2 hamsters compared with untreated TO-2 hamsters. Berlanga et al recently showed that GHRP-6 treatment attenuated the decrease in total SOD activity in a model of acute myocardial infarction.30 On the other hand, in the present study GSHPx activity did not differ among all 4 groups at 12 weeks of age. Although our findings suggest that antioxidant capacity might be relatively preserved in untreated TO-2 hamsters at 12 weeks of age, further enhancement of total SOD activity might in part contribute to the antioxidative effects of GHRP-2 in TO-2 hamsters. In addition, a recent study reported that ghrelin, a novel GHRP produced principally in the stomach, dose-dependently inhibited vascular NADPH oxidase activity and superoxide production in spontaneously hypertensive rats.31 We did not examine the effects of GHRP-2 on the production of ROS in the present study; however, GHRP-2 might have inhibitory effects on NADPH oxidase activity and superoxide production in common with ghrelin. Recently, Li et al reported that ghrelin inhibited proinflammatory cytokine production and nuclear factor-κB activation in human endothelial cells.32 Another study reported that hexarelin, one of the variants of GHRP, inhibited apoptosis in H9c2 cardiomyocytes and endothelial cells.33 Furthermore, Baldanzi et al reported that inhibition of apoptosis in H9c2 cardiomyocytes and endothelial cells by ghrelin was attributed to activation of extracellular signal-regulated kinase 1/2 and Akt serine kinases.34 These results suggest that various intracellular signaling pathways related to oxidative stress also contribute to the cardioprotective effects of GHRPs. One limitation of our study is that we were unable to show in this animal study whether the decreases in myocardial oxidative stress induced by GHRP-2 are the cause or result of amelioration of DCM. Further studies are required to elucidate the role of myocardial oxidative stress on the mechanism of cardioprotection by GHRP-2.

Effects of GHRP-2 on Progressive LV Remodeling and Dysfunction

Some investigators have reported beneficial cardiac effects of GHRP-2. Weekers et al previously demonstrated that GHRP-2 pretreatment selectively protected against the diastolic dysfunction of myocardial stunning in an isolated blood-perfused rabbit heart model.35 Recently, Xu et al reported that GHRP-2 administration improved LV function and remodeling in pressure-overload chronic heart failure rats, at least in part by suppressing stress-induced neurohormonal activations (such as norepinephrine, renin, angiotensin II, and aldosterone) and cardiomyocyte apoptosis.36 In addition, Furuta et al reported that GHRP-2 improved cardiac function in rat isolated hearts subjected to ischemia/reperfusion injury, independently of GH secretion.37 In TO-2 hamsters, the manifest cardiac myolysis occurred at the stage of initial LV dysfunction, probably as a result of myocardial ischemia and reperfusion caused by coronary microvascular abnormalities.11,12 In the present study, treatment with GHRP-2 attenuated the progression of LV dilation, wall thinning, interstitial fibrosis, and systolic dysfunction, as well as myocardial oxidative stress, in TO-2 hamsters at 12 weeks of age. These results suggest that the beneficial cardiac effects of GHRP-2 may be attributed to suppression of myocardial oxidative stress in TO-2 hamsters and that administration of GHRP-2 is a potential new approach to the treatment of DCM and heart failure. Our recent studies have shown that amelioration of progressive LV dysfunction in TO-2 hamsters by bisoprolol (β1-selective adrenoceptor...
blocker) and allopurinol (xanthine oxidase inhibitor) might stem in part from attenuation of the associated increases in myocardial oxidative stress.\(^{13,14}\)

We also found that treatment with either a low or a high dose of GHRP-2 ameliorated the decrease in the LV functional response to \(\beta\)-adrenergic stimulation in TO-2 hamsters at 12 weeks of age. Downregulation and desensitization of \(\beta\)-adrenergic receptor signaling is a major contributing factor in the contractile dysfunction of the failing heart. We recently reported that myocardial oxidative stress may be involved in the development of \(\beta\)-adrenergic desensitization in TO-2 hamsters.\(^{12}\) Furthermore, Haenen et al previously demonstrated that 4-HNE reduced the maximal response to \(\beta\)-adrenoceptor stimulation by isoproterenol in the heart.\(^{29}\) The present study showed that the concentration of 4-HNE was increased in the failing heart of untreated TO-2 hamsters. Ebina et al previously reported that oxidized catecholamine inhibited the type V adenyl cyclase (AC) activity, a major cardiac isoform, whereas the type II AC, a noncardiac isoform, was resistant to its inhibition in vitro.\(^{29}\) Because it is technically difficult to estimate the concentration of oxidized catecholamines in vivo, we could not measure the exact concentration of oxidized catecholamine in the myocardial synaptic cleft in our model. However, it is possible that myocardial oxidative stress might inhibit AC activity via oxidized catecholamine in the hearts of TO-2 hamsters. Therefore, the beneficial cardiac effects of GHRP-2 are likely attributable, at least in part, to the amelioration of the LV functional response to \(\beta\)-adrenergic stimulation by suppression of myocardial oxidative stress.

In the present study, we did find that treatment with GHRP-2 attenuated the progression of LV remodeling and dysfunction assessed by echocardiography in TO-2 hamsters. However, we did not perform a cardiac catheterization study, including measurements of LV dP/dt and LV end-diastolic pressure. Therefore, the absence of these data is a limitation of the current study for evaluating the hemodynamic mechanisms of GHRP-2 on cardiac function in detail.

**Conclusion**

Treatment with GHRP-2 ameliorated progressive LV remodeling and dysfunction in TO-2 hamsters independent of the systemic growth axis. Furthermore, GHRP-2 treatment suppressed the increase in the level of myocardial oxidative stress that accompanies the development of DCM in TO-2 hamsters. Our findings suggest that inhibition of myocardial oxidative stress by administration of GHRP-2 is a potential new approach to the treatment of DCM and heart failure.

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**Disclosure**

There are no conflicts of interest.

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