Comparison of Losartan and Carvedilol on Attenuating Inflammatory and Oxidative Response and Preserving Energy Transcription Factors and Left Ventricular Function in Dilated Cardiomyopathy Rats

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SUMMARY

We compared the effects of losartan and carvedilol on preserving left ventricular (LV) function in an experimental model of dilated cardiomyopathy (DCM) and examined the mechanisms of their pharmacological effects. The rats were divided into group 1 (normal control), group 2 (DCM), group 3 (DCM plus carvedilol 8 mg/kg/day bid orally), and group 4 (DCM plus losartan 20 mg/kg/day orally). All rats were sacrificed on day 90 following DCM induction. The results indicated that connexin43 protein expression and mRNA expressions of peroxisome proliferator-activated receptor-γ coactivator-1α, endothelial nitric oxide synthase, and interleukin-10 were significantly lower, whereas mRNA expressions of endothelin-1 and matrix metalloproteinase-9 were significantly higher in group 2 than in groups 1, 3, and 4 in LV myocardium (all \( P < 0.05 \)). Additionally, cytochrome C levels in LV myocardium and LV contractility were significantly lower, whereas fibrosis area, cellular apoptosis, and mitochondrial oxidative response of LV myocardium were significantly higher in group 2 than in groups 1, 3, and 4 (all \( P < 0.005 \)). In conclusion, losartan is comparable to carvedilol in attenuating inflammation, oxidative response, myocardial fibrosis and apoptosis, as well as in preserving energy transcription factors and LV function in DCM.  

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Key words: Dilated cardiomyopathy, Inflammation, Pharmacological modulation

DILATED cardiomyopathy (DCM), a common cause of congestive heart failure (CHF), is a leading cause of cardiogenic mortality worldwide.1-3) Most patients succumb to DCM either suddenly or due to progressive pump failure.3) Regard-
less of its etiology, systemic inflammatory response plays an important role in the pathogenesis of CHF. Additionally, elevated proinflammatory cytokines are known to damage the myocardium. Extensive research has demonstrated that increased inflammatory response is predictive of aggravated CHF severity and poor clinical outcome in various clinical settings.

Experimental studies have established close relationships between depressed energy transcription factors and cardiac function disturbances such as left ventricular (LV) dysfunction in DCM, diabetic cardiomyopathy, and myocardial infarction. Furthermore, changes in connexin43 (Cx43) expression patterns have been demonstrated to be associated with various cardiac pathologies and contribute to the initiation of cardiac arrhythmia. The integrity of Cx43 in DCM, however, has not been thoroughly investigated.

Growing evidence demonstrates that an increased level of oxidative stress resulting from excessive cardiac generation of reactive oxygen species contributes to endothelial and contractile dysfunction, myocyte apoptosis, and remodeling of the extracellular matrix in the heart. Superoxide production or proinflammatory cytokines are known to be increased in DCM patients.

Strategic management of symptomatic DCM patients has been investigated extensively. Among these regimens, medical therapy with angiotensin converting enzyme inhibitors (ACEIs) and beta-blockers is the most common treatment protocol and offers substantial benefits in improving LV function, CHF, and long-term survival. Bisoprolol and carvedilol therapies have recently been shown to significantly reduce oxidative stress in LV myocardium and skeletal muscle as well as preserve LV function in a DCM rat model. Surprisingly, while the pleiotropic effects of losartan have recently been recognized, little is known about the efficiency of losartan when compared with carvedilol in attenuating inflammation and oxidative stress, preserving Cx43 integrity between cardiomyocytes, and suppressing cellular apoptosis in DCM. Therefore, this study tested the hypothesis that losartan therapy is not inferior to carvedilol therapy in improving LV function, upregulating Cx43 protein expression in cardiomyocytes, and down-regulating cardiomyocyte apoptosis by attenuating inflammation, suppressing oxidative stress, and upregulating energy transcription factors in DCM rats.

**METHODS**

**Ethics, animals, and DCM model:** All experimental procedures were approved by the Institute of Animal Care and Use Committee at our hospital and performed according to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, National Academy Press, Washington, DC, USA, revised
Pathogen-free, adult male Sprague-Dawley (SD) rats weighing 250-300 g (Charles River Technology, BioLASCO Taiwan Co., Ltd., Taiwan) were utilized in this study. DCM was induced through immuno-reactive myocarditis as described previously. Briefly, 1 mg (0.1 mL) of porcine heart myosin (Sigma) was mixed with an equal volume of Freund complete adjuvant (Sigma) and injected into the footpad on day 1 and day 7. The rat model of DCM was then established after 5 weeks of immunization as previously documented.

**Randomization:** Ten healthy SD rats served as normal controls (group 1). DCM was induced in 30 SD rats which were then divided into group 2 (n = 10) (DCM only), group 3 (n = 10) (DCM treated with carvedilol: 8 mg/kg/day bid, orally) and group 4 (n = 10) (DCM treated by losartan: 20 mg/kg/day, orally). Both drugs were commenced on day 35 following DCM induction. All dosages were according to previous descriptions. The rats were sacrificed on day 90 following DCM induction.

**Functional assessment by echocardiography:** Transthoracic echocardiography was performed prior to DCM induction, and on day 35 as well as day 90 after DCM induction with a commercially available echocardiographic system (UF-750XT) equipped with an 8-MHz linear-array transducer for animals (FUKUDA Denshi Co., Tokyo) with the rats in a supine position and under general anesthesia (chlo-ral hydrate 35 mg/kg i.p.). The heart was imaged in 2-dimensional mode in the short-axis view of the left ventricle at the level of the papillary muscle. This view was used to record M-mode tracings. The LV internal dimensions [end-systolic diameter (ESD) and end-diastolic diameter (EDD)] were measured according to the American Society of Echocardiography leading-edge method using at least 3 consecutive cardiac cycles. The LV ejection fraction (LVEF) was calculated as follows:

\[
\text{LVEF} \, (\%) = \left[\frac{(LVEDD^3 - LVEDS^3)}{LVEDD^3}\right] \times 100
\]

All measurements were performed by an animal cardiologist blind to the treatment and nontreatment groups.

**Hemodynamic studies:** On day 90 after DCM induction, all rats were anesthetized by intraperitoneal injections of chloral hydrate (35 mg/kg). After shaving the chest, each animal underwent endotracheal intubation with positive-pressure ventilation (180 mL/minute) with room air using a Small Animal Ventilator (SAR-830/A, CWE Inc., Boston, USA). The heart was exposed by left thoracotomy. A sterile 20-gauge needle, which was connected to a hemodynamic monitor (Hewlett M1165A, Packard Model 56s), was inserted into the left ventricle of each animal to measure LV systolic blood pressure. The rats were then euthanized, and their hearts harvested, weighed, and divided into 3 portions which were fixed in 10% buffered formalin, cryopreserved with OCT, and frozen in liq-
uid nitrogen and then stored at -80°C for later use, respectively.

**Immunolabeling of connexin43 and quantitative image data analysis:** Six serial sections of LV myocardium (3 longitudinal and 3 transverse) of 4 µm thickness were prepared using a Cryostat (Leica CM3050S) for Cx43 immunolabeling. To colocalize troponin I and Cx43 in the same sample, tissue sections were first incubated with a mixture of polyclonal anti-Cx43 (1:200) plus anti-troponin I (1:200) for 24 hours at 4°C and then incubated with anti-mouse FITC (1:200) and anti-rabbit rhodamine (1:200) for 30 minutes at room temperature.

The integrated area (µm²) of Cx43 spots in the slides was calculated using Image Tool 3 (IT3) image analysis software (University of Texas, Health Science Center, San Antonio, TX, UTHSCSA; Image Tool for Windows, Version 3.0, USA) as described elsewhere.²⁹ For each animal, 3 sections were selected for quantification. Each section was analyzed under 3 randomly selected high-power fields (HPFs) (400 ×). After determining the number of pixels in each Cx43 spot per HPF, the total number of pixels in the 3 HPFs in each section was determined. This procedure was repeated in two other sections for each animal. The mean number of pixels per HPF for each animal was then determined by summing all pixel numbers and dividing by 9. The mean area of Cx43 per HPF was obtained by adopting a conversion factor of 19.24 (1µm² representing 19.24 pixels).

**Histological study of fibrosis area:** Masson Trichrome staining was used for assessing fibrosis in LV myocardium. Calculation of the integrated area (µm²) of fibrosis in LV myocardium in the sections was identical to that in Cx43 quantification using Image Tool 3 (IT3) image analysis software.

**Isolation of mitochondria:** The LV myocardium was excised and washed with buffer A (100 mM Tris-HCl, 70 mM sucrose, 10 mM EDTA and 210 mM mannitol, pH 7.4). Samples were finely minced in cold buffer A and then incubated for 10 minutes. All samples were homogenized in an additional 3 mL of buffer A using a motor-driven grinder. The homogenate was centrifuged twice at 700 g for 10 minutes at 4°C. The supernatant was again centrifuged at 8,500 g for 15 minutes, and the pellets were then washed with buffer B (10 mM Tris-HCl, 70 mM sucrose, 1 mM EDTA, and 230 mM mannitol, pH 7.4). The mitochondria-rich pellets were then collected and stored at -70°C.

**Western blot analysis for Cx43 and cytochrome C in mitochondria:** Equal amounts (10-30 µg) of protein extracts from remote viable LV myocardium were loaded and separated by SDS-PAGE using 8-10% acrylamide gradients. Molecular weight standards and rat brain extracts rich in PKC-ε were electrophoresed as controls. Following electrophoresis, the separated proteins were transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, USA). Nonspecific proteins were blocked by incubating the membrane in blocking buffer (5% nonfat dry milk in T-TBS containing 0.05% Tween
overnight. The membranes were incubated with the indicated primary antibodies Cx43, 1: 1000, Chemicon; Cytochrome C, 1: 1000, BD Biosciences, USA; Actin, 1:10000, Chemicon) for 1 hour at room temperature. Horseradish peroxidase-conjugated anti-mouse immunoglobulin IgG (1:2000, Amersham Biosciences) was applied as the second antibody for 1 hour at room temperature. The washing procedure was repeated 8 times in 1 hour, and immunoreactive bands were visualized by enhanced chemiluminescence (ECL) (Amersham Biosciences) and exposure to Biomax L film (Kodak, USA). For quantification, digitized ECL signals were analyzed using Labwork UVP software.

**Real-time quantitative PCR analysis:** Changes in mRNA expression of endothelin-1 (ET-1), matrix metalloproteinase-9 (MMP-9), IL-10, endothelial nitric oxide synthetase (eNOS), and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) were analyzed using real-time polymerase chain reaction (RT-PCR) that was conducted using Light Cycler TaqMan Master (Roche, Germany) in a single capillary tube according to the manufacturer’s guidelines for individual component concentrations. Forward and reverse primers were each designed using sequences from different exons of the target gene to eliminate the possibility of amplifying genomic DNA.

During PCR, the probe was hybridized to its complementary single-strand DNA sequence within the PCR target. As amplification occurred, the probe was degraded due to the exonuclease activity of Taq DNA polymerase, thereby separating the quencher from reporter dye during extension. During the entire amplification cycle, light emission increased exponentially. A positive result was identified as the threshold cycle value at which reporter dye emission appeared above background.

**Oxidative stress reaction of LV myocardium:** An Oxyblot Oxidized Protein Detection Kit was purchased from Chemicon (S7150). A DNPH derivatization was performed for 15 minutes according to the manufacturer’s instructions on 6 µg of protein. One-dimensional electrophoresis was carried out on 12% SDS/polyacrylamide gel after DNPH derivatization. Proteins were transferred to nitrocellulose membranes. The membrane was incubated in the primary antibody solution (anti-DNP 1: 150) for 2 hours followed by incubation in a second antibody solution (1: 300) for 1 hour at room temperature. The washing procedure was repeated 8 times within 40 minutes. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL; Amersham Biosciences) and then exposed to Biomax L film (Kodak). For quantitation, ECL signals were digitized using Labwork software (UVP). A standard control sample was always loaded on each gel.

**TUNEL assay for apoptotic nuclei:** In situ DNA fragments were detected by the TUNEL method. Sections were analyzed by an in situ Cell Death Detection Kit,
AP (Roche) according to the manufacturer’s guidelines. For each rat, 6 sections (3 longitudinal and 3 transverse sections of LV myocardium) were used for the TUNEL assays. The TUNEL-positive cells were examined in 3 randomly chosen HPFs (× 400) and normalized to the total number of cells divided by 18.

![Image of bar graph showing mean fibrotic area (µm²)/high-power field (HPF) of left ventricular (LV) myocardium in each group of rats on day 90 following dilated cardiomyopathy (DCM) induction (n = 7). * versus †, P = 0.01; * versus ‡, P = 0.025; ‡ versus †, P = 0.040. Lower Panel: No fibrosis was observed in normal control (A). Markedly increased fibrosis area (white arrows) in DCM group (B) compared with that in DCM + Carvedilol (C) and DCM + Los (D) groups. Scale bars in right lower corner represent 50 µm (A, B, C and D).]
**Statistical analysis:** Data are expressed as the mean ± SD. The significance of differences between two groups was evaluated by the t-test. Continuous variables among the 4 groups were compared using the Kruskal-Wallis test followed by multiple comparison procedure using the Wilcoxon rank sum test and Bonferroni correction. Statistical analysis was performed using SAS statistical software for Windows, version 8.2 (SAS Institute, Cary, NC). A probability value < 0.05 was considered statistically significant.

**RESULTS**

**Mortality in each group:** There were no deaths in group 1 during the study period, but there were 3, 2, and 2 deaths in groups 2, 3, and 4, respectively. There was no difference in the mortality rate among the 4 groups (P = 0.456) when analyzed using the Fischer exact test.

**Fibrosis of LV myocardium (Figure 1):** Masson Trichrome staining revealed no fibrotic tissue in group 1 (normal control). However, the mean area of fibrotic tissue was significantly higher in group 2 (DCM only) than in group 3 (DCM plus carvedilol-treated) and group 4 (DCM plus losartan-treated) and significantly higher in group 4 than in group 3.

### Table. Summary of Cardiac and Hemodynamic Parameters of the Study Animals

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3*</th>
<th>Group 4*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (gm)</td>
<td>367.5 ± 18.4</td>
<td>363.5 ± 17.9</td>
<td>366.8 ± 16.1</td>
<td>362.9 ± 16.6</td>
<td>0.890</td>
</tr>
<tr>
<td>Final body weight (gm)</td>
<td>540.3 ± 44.8</td>
<td>536.8 ± 32.9</td>
<td>531.9 ± 35.2</td>
<td>524.2 ± 35.3</td>
<td>0.764</td>
</tr>
<tr>
<td>Final heart weight (gm)</td>
<td>1.23 ± 0.04*</td>
<td>1.62 ± 0.11b</td>
<td>1.41 ± 0.04cd</td>
<td>1.37 ± 0.06ed</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Initial LVEF (%)</td>
<td>92.8 ± 2.50</td>
<td>92.8 ± 2.70</td>
<td>93.1 ± 2.52</td>
<td>93.1 ± 2.44</td>
<td>0.977</td>
</tr>
<tr>
<td>Initial LVEDD (mm)</td>
<td>0.75 ± 0.09</td>
<td>0.74 ± 0.04</td>
<td>0.73 ± 0.04</td>
<td>0.74 ± 0.05</td>
<td>0.834</td>
</tr>
<tr>
<td>Initial LVESD (mm)</td>
<td>0.29 ± 0.02</td>
<td>0.28 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>0.914</td>
</tr>
<tr>
<td>Day-35 LVEF (%)</td>
<td>92.9 ± 3.10a</td>
<td>85.5 ± 2.80e</td>
<td>85.0 ± 2.04b</td>
<td>85.1 ± 2.46b</td>
<td>0.009</td>
</tr>
<tr>
<td>Day-35 LVEDD (mm)</td>
<td>0.73 ± 0.04a</td>
<td>0.82 ± 0.04a</td>
<td>0.82 ± 0.02b</td>
<td>0.81 ± 0.04b</td>
<td>0.004</td>
</tr>
<tr>
<td>Day-35 LVESD (mm)</td>
<td>0.31 ± 0.03a</td>
<td>0.40 ± 0.04a</td>
<td>0.42 ± 0.02b</td>
<td>0.42 ± 0.03b</td>
<td>0.0008</td>
</tr>
<tr>
<td>Day-90 LVEF (%)</td>
<td>93.0 ± 2.32a</td>
<td>82.4 ± 1.52e</td>
<td>88.0 ± 2.58c</td>
<td>87.4 ± 1.82c</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day-90 LVEDD (mm)</td>
<td>0.74 ± 0.07a</td>
<td>0.87 ± 0.02a</td>
<td>0.81 ± 0.02a</td>
<td>0.81 ± 0.03a</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day-90 LVESD (mm)</td>
<td>0.30 ± 0.01a</td>
<td>0.48 ± 0.02a</td>
<td>0.41 ± 0.02a</td>
<td>0.41 ± 0.02a</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LVSBP (mmHg)</td>
<td>88.7 ± 4.2a</td>
<td>83.6 ± 3.9a</td>
<td>84.3 ± 3.3b</td>
<td>86.4 ± 4.2ab</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. LVEF indicates left ventricular ejection fraction; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; LVSBP, left ventricular systolic blood pressure; * Group 1, normal control; Group 2, dilated cardiomyopathy (DCM); Group 3, DCM plus carvedilol treatment; and Group 4, DCM plus losartan treatment.

Means with difference letters (*, a–c) indicate significant difference (at 0.05 level) using the Wilcoxon rank sum test with Bonferroni correction.
Figure 2.
A. Upper Panel: Integrated area ($\mu m^2$) of Connexin43 (Cx43)/HPF (400 ×) from sections of LV myocardium in each group of rats on day 90 following DCM induction ($n = 7$). * versus †, $P = 0.0007$; * versus ‡, $P < 0.02$; ‡ versus †, $P < 0.04$.
Lower Panel: Semiquantitative immunofluorescence imaging study under microscope (400 ×) identified fewer spots of Cx43-positive staining (white arrows) located between cardiomyocytes in DCM rats (group 2) than in normal (group 1), DCM + Car (group 3), and DCM + Los (group 4) animals. Note the inconspicuous Cx43 gap junctions and its reduction in number in group 2 compared to groups 1, 3, and 4. Scale bars in right lower corner represent 50 $\mu m$.

B. Upper Panel: Western blot analyses of Cx43 protein expression in each group of rats on day 90 following DCM induction. Notably lower Cx43 protein expression in DCM rats (group 2) than in normal control (group 1), DCM + Car (group 3), and DCM + Los (group 4) animals.
Lower Panel: Cx43 protein expression in the LV myocardium in each group of rats on day 90 following DCM induction ($n = 7$). * versus †, $P = 0.0213$; † versus ‡, $P < 0.047$; * versus ‡, $P > 0.5$. 
Final body weight, heart weight, and serial echocardiographic findings (Table): There was no significant difference in the initial or final body weight among the 4 groups. In addition, the final heart weight did not differ among the 4 groups, and there were no significance differences in initial LVEF, LVEDD, or LVESD. On day 35 following DCM induction, however, LVEF was significantly lower and LVEDD and LVESD were significantly elevated in groups 2, 3, and 4 compared to group 1, whereas the LVEF, LVEDD and LVESD did not differ among groups 2, 3, and 4. Moreover, by day 90, the LVEF was significantly lower, whereas LVEDD and LVESD were significantly higher in group 2 than in groups 1, 3, and 4. LVEF was significantly lower and LVEDD and LVESD were significantly higher in groups 3 and 4 than in group 1.

Connexin43 expression of LV: Figure 2A (upper panel) shows the quantification results of the integrated area (µm²) of clustered Cx43 spots in LV myocardium for each group on day 90 following DCM induction. The summation area of Cx43 did not significantly differ between groups 3 and 4, whereas the summation area of Cx43 spots was substantially lower in group 2 than in groups 1, 3, and 4 and significantly higher in group 1 than in groups 3 and 4. In addition, the number of intact Cx43 gap junctions and its distribution were more homogenous in groups 1, 3, and 4 than in group 2 (Figure 2A: lower panel).

Western blotting of Cx43 in LV (Figure 2B): The Cx43 protein expression of the left ventricle was substantially lower in group 2 than in groups 1, 3, and 4 and significantly lower in groups 3 and 4 than in group 1 on day 90 after DCM induction.

RT-PCR analysis of ET-1, MMP-9, IL-10, eNOS, and PGC-1α mRNA expressions (Figure 3): On day 90 following DCM induction, the mRNA expressions of ET-1 and MMP-9 were significantly elevated in group 2 compared with that in groups 1, 3, and 4 and significantly higher in groups 3 and 4 than in group 1 (Figure 3A). Conversely, the mRNA expressions of IL-10, eNOS (Figure 3A), and PGC-1α (Figure 3B) were significantly reduced in group 2 compared with that in groups 1, 3, and 4, and were significantly lower in groups 3 and 4 than in group 1. Moreover, the PGC-1α mRNA expression was significantly higher in group 4 than in group 3 (Figure 3B).

Cytochrome C protein expression in mitochondria and cytosol (Figure 4): The total cytochrome C protein expression in mitochondria was significantly lower in group 2 than in groups 1, 3, and 4. Conversely, the total amount of cytochrome C protein expression in cytosol was significantly higher in group 2 than in groups 1, 3, and 4. These findings indicated that the expression of cytochrome C, an index of energy supply and storage in mitochondria, was more suppressed in group 2 than in the other groups, suggesting significant mitochondrial damage in the myocardium of this group of animals.
Figure 3.
A. a: Endothelin-1 (ET-1) mRNA expression in each group of rats on day 90 following DCM induction (n = 7). † versus *, P < 0.0001; † versus ‡, P < 0.04; ‡ versus *, P < 0.001.
b: Matrix metalloproteinase (MMP)-9 mRNA expression in each group of rats on day 90 following DCM induction (n = 7). † versus *, P = 0.0025; † versus ‡, P < 0.04; ‡ versus *, P < 0.015.
c: Endothelial nitric oxide synthase (eNOS) mRNA expression in each group of rats on day 90 following DCM induction (n = 7). * versus †, P = 0.01; * versus ‡, P = 0.038; ‡ versus †, P < 0.047.
d: Interleukin (IL)-10 mRNA expression in each group of rats on day 90 following DCM induction (n = 7). * versus †, P < 0.0001; * versus ‡, P < 0.001; † versus ‡, P < 0.019.
B. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA expression in each group of rats on day 90 following DCM induction (n = 7).
* versus †, ‡, or ¶, P < 0.0001; † versus ‡, P < 0.001; ‡ versus ¶, P = 0.018; ¶ versus †, P = 0.046.
Figure 4.
A. Upper Panel: Western blot analyses of mitochondrial cytochrome C protein expression in each group of rats on day 90 following DCM induction. Note the substantially lower cytochrome C protein expression in DCM (group 2) than in other groups.

Lower Panel: Western blots of mitochondrial cytochrome C protein expression of LV myocardium in each group of rats on day 90 following DCM induction (n = 7). * versus †, P < 0.006.

B. Upper Panel: Western blot analyses of cytosolic cytochrome C protein expression using anti-DNP antibody (1: 150) in each group of rats on day 90 following DCM induction. Note remarkably higher cytochrome C protein expression in DCM (group 2) than in normal control (group 1), DCM + Car (group 3), and DCM + Los (group 4), and higher expression in groups 3 and 4 than in group 1.

Lower Panel: Western blots of mitochondrial cytochrome C protein expression in LV myocardium of each group on day 90 following DCM induction (n = 7). † versus *, P = 0.002; † versus ‡, P < 0.005; ‡ versus *, P < 0.044.

Figure 5.
A. Upper Panel: Notably increased density of oxidative index in DCM rats (group 2) compared to that in normal control (group 1), DCM + Car (group 3) and DCM + Los (group 4) animals.

Lower Panel: Oxidative response of LV myocardium in each group of rats on day 90 following DCM induction (n = 7). † versus *, P = 0.006; † versus ‡, P = 0.017; † versus ¶, P = 0.114; ¶ versus *, P = 0.045; ¶ versus †, P = 0.181; ‡ versus *, P = 0.072.

B. TUNEL staining of apoptotic nuclei in LV myocardium on day 90 following DCM induction (n = 7). † versus * and ¶, P < 0.0001; ‡ versus *, P < 0.001.
Intensity of oxidative stress (Figure 5): Western blot analysis revealed no differences in the mitochondrial oxidative response between groups 2 and 4 as well as between groups 3 and 4. However, the oxidative response was significantly higher in group 2 than in groups 1 and 3, and significantly higher in group 4 than in group 1 (left panel of Figure 5A).

Apoptotic nuclei formation in remote viable myocardium (Figure 5): The right panel of Figure 5B shows the results of quantitative TUNEL detection of apoptotic nuclei. The results showed that the number of apoptotic nuclei was significantly elevated in group 2 compared with that in groups 1, 3, and 4 and significantly higher in groups 3 and 4 than in group 1.

DISCUSSION

Alternation in energy metabolism is one of the key mechanisms underlying functional defects such as decreased LV contractility.\(^\text{10}\) In addition, expression of PGC-1\(\alpha\), which is a transcriptional coactivator and primary regulator of oxidative metabolism and mitochondrial biogenesis, is suppressed in CHF.\(^\text{10}\) Interestingly, our recent studies have also shown that PGC-1\(\alpha\) gene expression is significantly reduced in DM rats with LV dysfunction and in rats with acute myocardial infarction (AMI).\(^\text{11,12}\) In the present study, one of the important findings was that PGC-1\(\alpha\) gene expression was remarkably suppressed in DCM rats. Moreover, the cytochrome C level in mitochondria was also markedly decreased in the DCM rats. Furthermore, LV function was substantially impaired in DCM rats compared with normal controls. Our findings, therefore, corroborate those of previous experimental studies.\(^\text{10-12}\) Of interest in the present study is that losartan was comparable to carvedilol in preserving LV function and cytochrome C levels in mitochondria. Additionally, losartan was found to be superior to carvedilol in inhibiting PGC-1\(\alpha\) gene down-regulation in induced DCM. A recent study\(^\text{29}\) reported that some angiotensin II type I (AT1) receptor blockers may act as partial agonists of peroxisome proliferator activated receptor-\(\gamma\). Therefore, our findings, in addition to extending those of a previous study,\(^\text{10}\) further strengthen the results of recent investigations from other authors\(^\text{29}\) and those of our more recent studies,\(^\text{11,12}\) that revealed down-regulation of PGC-1\(\alpha\) gene expression in both DM and AMI rats.

Free radical production and proinflammatory cytokines are known to increase in the clinical settings of DCM and CHF of various etiologies.\(^\text{6-8,10,16-19}\) The links among increased reactive oxygen species and endothelial damage, LV dysfunction, cellular apoptosis, and remodeling of the extracellular matrix in the
heart are well established.\textsuperscript{10,16-18} Another important finding in the current study was that, compared with the control group, the DCM rats had a notably higher level of oxidative stress which was significantly improved after carvedilol therapy. Recent studies have shown that either bisoprolol or carvedilol therapy significantly attenuates oxidative stress in LV myocardium and skeletal muscle and improves heart function in an animal model of DCM.\textsuperscript{10,18,23,24} The present study also demonstrated consistent findings. Interestingly, the therapeutic efficacy of losartan was inferior to carvedilol in terms of oxidative stress reduction in the current study, although the difference was not statistically significant. This result suggests that losartan has limited efficacy in suppressing reactive oxygen species production in the myocardium of DCM rats.

Experimental studies demonstrate that numerous proinflammatory cytokines and C-reactive proteins (CRP) directly participate in the damage of endothelial cells and myocardium.\textsuperscript{7,8,27,30,31} Clinical studies have further shown that increased circulating levels of inflammatory biomarkers are predictive of untoward clinical outcomes of patients with various cardiovascular diseases.\textsuperscript{4-7,19} The most important finding in the present study is that the mRNA expressions of MMP-9 and ET-1 were significantly higher, whereas those of IL-10, an anti-inflammatory cytokine, and eNOS were significantly suppressed in DCM than in normal control rats. In addition, LV performance and Cx43 expression were significantly lower, whereas cardiomyocyte apoptosis was significantly higher in DCM rats than in normal controls. Therefore, the results of the present study reinforce the findings of previous studies\textsuperscript{7,8,27,30,31} that persistent inflammation elicited by broad arrays of proinflammatory cytokines and inflammatory proteins may play an essential role in depressing LV function.

Treating CHF resulting from DCM is an important clinical issue. Recent studies have discussed the efficiency of beta-blocker treatment on DCM.\textsuperscript{18,21,22} Interestingly, in this study, proinflammatory cytokines were markedly attenuated, and both IL-10 and eNOS, indexes of anti-inflammatory markers, were significantly up-regulated after treatment with either carvedilol or losartan. While the effectiveness of these two medical therapeutic strategies is equally promising, the anti-inflammatory effect of carvedilol on treating DCM requires further elucidation. Recent experimental investigations\textsuperscript{18,23,24} have demonstrated that bisoprolol and carvedilol possess antioxidative effects. These findings,\textsuperscript{18,23,24} together with those of recent studies demonstrating the anti-inflammatory properties of losartan,\textsuperscript{25,26,30} may at least partly explain the results of the present study.

This study has several limitations. First, we did not test the possible dose-related effects of each drug. Therefore, whether losartan 20 mg/kg/day or
carvedilol 8 mg/kg/day bid is the optimal dosage for the rats was unknown. However, the dosage of each drug was based on recommendations from previous studies.\textsuperscript{1,2,28} Second, since it is difficult to compare the overall therapeutic effects between carvedilol and losartan because of their discrepancies in anti-inflammatory, antioxidative, and antiapoptotic effects, it is unclear whether the dosage of these two drugs used in this study is comparable.

In conclusion, either losartan or carvedilol therapy can significantly preserve LV function, up-regulate Cx43 expression in cardiomyocytes and down-regulate cellular apoptosis in an animal model of DCM by attenuating inflammation and oxidative stress of myocardium and upregulating energy transcription factors.

REFERENCES

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