Importance of antioxidant and antiapoptotic effects of β-receptor blockers in heart failure therapy

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Kawai, Keisuke, Fuzhong Qin, Junya Shite, Weike Mao, Shuji Fukuoka, and Chang-seng Liang. Importance of antioxidant and antiapoptotic effects of β-receptor blockers in heart failure therapy. Am J Physiol Heart Circ Physiol 287: H1003–H1012, 2004. First published April 22, 2004; 10.1152/ajpheart.00797.2003.—The present study was carried out to determine whether beneficial effects of carvedilol in congestive heart failure (CHF) are mediated via its β-adrenergic blocking, antioxidant, and/or α-adrenergic blocking action. Rabbits with heart failure induced by rapid cardiac pacing were randomized to receive subcutaneous carvedilol, metoprolol, propranolol plus doxazosin, or placebo pellets for 8 wk and compared with sham-operated rabbits without pacing. We found rapid cardiac pacing produced clinical heart failure, left ventricular dilation, and decline of left ventricular fractional shortening. This was associated with an increase in left ventricular end-diastolic pressure, decrease in left ventricular first derivative of left ventricular pressure, and myocyte hypertrophy. Tissue oxidative stress measured by GSH/GSSG was increased in the heart with increased oxidation product of mitochondrial DNA, 8-oxo-7,8-dihydro-2′-deoxyguanosine, increase of Bax, decrease of Bel-2, and increase of apoptotic myocytes as measured by anti-single-stranded DNA monoclonal antibody. Administration of carvedilol and metoprolol, which had no effect in sham animals, attenuated cardiac ventricular remodeling, cardiac hypertrophy, oxidative stress, and myocyte apoptosis in CHF. In contrast, propranolol plus doxazosin, which has less antioxidant effects, produced smaller effects on left ventricular function and myocyte apoptosis. In all animals, GSH/GSSG correlated significantly with changes of left ventricular end-diastolic pressure (r = -0.678, P < 0.0001), fractional shortening (r = 0.706, P < 0.0001), and apoptotic myocytes (r = -0.473, P = 0.0001). Thus our findings suggest antioxidant and antiapoptotic actions of carvedilol and metoprolol are important determinants of clinical beneficial effects of β-receptors in the treatment of CHF.

carvedilol; metoprolol; oxidative stress; myocyte apoptosis; myocyte hypertrophy.

RECENT STUDIES HAVE SHOWN that β-adrenergic receptor blockers such as carvedilol, metoprolol, and bisoprolol are efficacious in the treatment of congestive heart failure (CHF) (3). These agents not only increase left ventricular systolic function (15, 28, 30) but also reduce cardiac mortality and morbidity in patients with CHF (7, 23, 31–33). However, the extent of improvements produced by these agents varies, with the greatest improvement in mortality with carvedilol (31). In a direct comparison study published recently (33), carvedilol was shown to produce a greater survival benefit in patients with CHF than metoprolol. Whereas the discrepancies among the studies may relate to the difference in patient populations or degree of β-receptor blockade, these β-adrenergic blockers may have additional properties that contribute to their different clinical benefits. Unlike metoprolol and bisoprolol, which are selectively β1-adrenergic receptor blockers, carvedilol is a nonspecific blocker that inhibits both β1- and β2-adrenergic receptors (39). In addition, carvedilol exhibits α-adrenergic blocking and potent antioxidant properties (51). This antioxidant effect of carvedilol may be clinically important because oxygen free radicals, which have been shown to increase in heart failure (43), can cause myocyte apoptosis (4), myocardial β-adrenergic receptor signal transduction abnormalities (21), and contractile dysfunction (6). In a recent study (35), we report that left ventricular mechanical function improved with antioxidant vitamins in animals with tachypacing-induced cardiomyopathy. In addition, antioxidant vitamins reduced the proapoptotic-to-antiapoptotic protein ratio and myocyte apoptosis in the CHF animals (42). Antioxidant vitamins also have been shown to decrease myocyte apoptosis and restore the function of myocardial β-adrenergic receptor-coupled signal transduction pathway in norepinephrine-induced cardiomyopathy (21, 34). In this study, we proposed to determine whether carvedilol produced antioxidant effects in pacing-induced cardiomyopathy. We measured left ventricular function by serial echocardiography. Myocardial oxidative stress was measured by the GSH-to-GSSG ratio (GSH/GSSG) and mitochondrial DNA (mtDNA) 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG). 8-oxo-dG is a sensitive, stable marker of oxidative stress in cellular DNA (9). We also measured the proapoptotic Bcl-2 and proapoptotic Bax proteins by Western blot, myocyte apoptosis using the anti-single-stranded DNA monoclonal antibody (47), and cardiac myocyte size (34). In addition, to study the relative roles of antioxidant, nonspecific β-adrenergic blocking, and α-adrenergic properties of carvedilol, we included two other groups of CHF animals treated with either metoprolol, a β1-selective blocking agent, or a combination of propranolol plus doxazosin, which block both α- and β-adrenergic receptors with little antioxidant effect (1). These three groups of animals were compared with animals treated with placebo. Our results indicate that carvedilol and metoprolol exert a greater beneficial effect than propranolol plus doxazosin. Our findings suggest the antioxidant property of β-blockers is clinically important in the treatment of heart failure.

MATERIALS AND METHODS

Animal model. The study was approved by the University of Rochester Committee on Animal Resources and conformed to the

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guiding principles approved by the Council of the American Physiological Society and the National Institutes of Health “Guide on Humane Care and Use of Laboratory Animals.”

Adult, healthy New Zealand White rabbits (2.8–3.5 kg, 4–6 mo of age) were chosen for production of experimental heart failure using a modified rapid cardiac pacing technique (16). Under general isoflurane anesthesia, subxiphoid thoracotomy and pericardiotomy were performed for placement of two shielded pacing leads (model TPW 50; Ethicon, Somerville, NJ) onto the left ventricular apex and the left pectoral muscle, respectively. One week after surgery, rabbits were randomly assigned to receive either pacing at a rate of 340 beats/min with a model 8086 Prevail implantable programmable pacemaker modified for rapid pacing (Medtronic, Minneapolis, MN) (CHF animals) or no cardiac pacing (sham animals). The animals developed progressive heart failure beginning 1–2 wk after start of rapid ventricular pacing (16) and evidence of oxidative stress and myocyte apoptosis after 8 wk of pacing (35).

Experimental protocol. Animals with CHF and sham animals were each divided into four groups according to their drug regimens: 1) carvedilol (1 mg·kg⁻¹·day⁻¹), 2) metropolol (2 mg·kg⁻¹·day⁻¹), 3) propranolol (3 mg·kg⁻¹·day⁻¹) plus doxazosin (0.5 mg·kg⁻¹·day⁻¹), and 4) placebo. The drugs were delivered at the specified doses over 8 wk using subcutaneous pellets (Innovative Research of America, Sarasota, FL). Doses of the medications were chosen to produce similar β-adrenoceptor blockade on the basis of their relative potencies. Doses of carvedilol and metropolol chosen for the study were also equivalent to the clinical therapeutic doses used in two large human clinical trials (23, 31). Animals were examined weekly for clinical evidence of heart failure and echocardiographic changes of left ventricular mechanical function in a lightly anesthetized state using katanime (20 mg/kg) and midazolam (0.6 mg/kg). Immediately after cardiac pacing was discontinued at 8 wk, the animals were anesthetized for measuring arterial blood norepinephrine by HPLC (42) and resting hemodynamics. The animals were then killed with intravenous pentobarbital sodium (42) and resting hemodynamics. The animals were then killed with intravenous pentobarbital sodium (>100 mg/kg). The heart was removed, weighed, and rinsed in ice-cold oxygenated normal saline. Ventricles were separated from septum and weighed. Transmural samples from left ventricles were processed immediately or stored in liquid nitrogen for later analysis.

Echocardiographic and hemodynamic measurements. Two-dimensional and M-mode echocardiography were obtained by using a 5-MHz transducer on a Toshiba model SSH-60A sonographic system (Toshiba America Medical Systems, Tustin, CA). Maximal left ventricular end-diastolic dimension (EDD) and end-systolic dimension (ESD) were measured and used to calculate left ventricular fractional shortening (FS) by the following equation: FS = [(EDD – ESD)/EDD] × 100.

For hemodynamic studies, animals were anesthetized with ketamine (35 mg/kg) and midazolam (0.8 mg/kg). A saline-filled catheter was inserted into the left jugular vein. A 20-gauge saline-filled catheter (Insyte; Deseret Medical, Becton-Dickinson, Sandy, UT) was introduced into the aorta through the left carotid artery and connected to a pressure transducer. A 2-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was placed into the left ventricle through the right carotid artery for measuring left ventricular pressure. Electrocardiogram, aortic pressure, and the first derivative of left ventricular pressure (dP/dt) were recorded on a Brush model 480 multichannel recorder (Gould, Cleveland, OH). Resting hemodynamic measurements were obtained in triplicate over a 20-min steady-state period at least 30 min after the catheterization. Averages of triplicate measurements were used for statistical analyses. To determine the relative efficacies of the three β-adrenergic receptor blockers on the myocardial β-adrenergic receptors, we also measured the peak heart rate and left ventricular dP/dt responses after an intravenous bolus dose (0.4 μg/kg) of isoproterenol in the sham-operated animals treated with placebo, carvedilol, metropolol, or propranolol plus doxazosin.

Myocardial GSH measurement. Fresh left ventricular myocardial tissue was homogenized in three volumes of 1% picric acid, and the supernatant was collected for measuring total GSH using a GSH reductase-coupled enzymatic assay (13) on a Lambda 3 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, CT). Total GSH was calculated from a standard curve of purified GSH, and GSSG was measured by masking the reduced GSH with 2-vinyl pyridine in the enzymatic assay. GSH/GSSG was calculated.

Myocardial mtDNA 8-oxo-dG measurement. Left ventricular myocardium was prepared for isolation of mitochondria (45), extracted using a QIAamp blood kit (Qiagen; Valencia, CA) per the manufacturer’s instructions and digested into deoxynucleosides by nuclease P₁ and alkaline phosphatase. The deoxynucleoside mixture was filtered through a 0.22-μm nylon filter and injected into an YMC Basic S3 μ 4.6 × 150 mm column (Water; Milford, MA) in a BAS 480 HPLC system (Bioanalytical System; West Lafayette, IN), with a mobile phase of 5% methanol in 100 mM lithium acetate buffer (pH 5.2). Detection of 8-oxo-dG and 2′-deoxyguanosine (dG) was performed on a model 5200A Couloumbo II electrochemical detector equipped with a model 5011 analytical cell and model 5021 guard cell (Environmental Science Associates; Chelmsford, MA). Purified 8-oxo-dG (Environmental Science Associates) and dG (Sigma-Aldrich) were used for calibration.

Myocyte apoptosis by monoclonal antibody to single-stranded DNA. Frozen left ventricular tissue was prepared for myocyte apoptosis measurement as described previously (34). Briefly, the tissue sections were fixed in 85% methanol in phosphate-buffered saline and incubated with mouse anti-single-stranded DNA monoclonal antibody (Chemicon International; Temecula, CA), biotinylated anti-mouse IgM (Vector Laboratory; Burlingame, CA), and avidin and biotinylated horseradish peroxidase macromolecular complex (Vector laboratory). The sections were stained with 3-amo-no-9-ethycarbazole (Vector Laboratory) and hematoxylin (Vector laboratory). Primary antibody was omitted as negative control. For the positive control, sections were first incubated with proteinase K (20 mg/ml) to induce apoptotic changes (47). Samples were analyzed under a light microscope. The number of apoptotic myocyte nuclei was scored per 10,000 myocytes.

Cardiac myocyte size by electron microscopic morphometry. Left ventricular muscle specimens were prepared for electron microscopic examination of cardiac myocytes (34). Muscle samples were first fixed in 2% glutaraldehyde and 4% paraformaldehyde in phosphate-buffered saline and then postfixed in 1.0% osmium tetroxide and embedded in Epon epoxy resin. Blocks were sectioned and stained with toluidine blue for light microscopic evaluation. Ultrathin sections were stained with uranyl acetate and lead citrate for examination on a Hitachi 7100 transmission electron microscope. Images of longitudinal and cross-sectional fibers were digitized from an original electron microscopic magnification of ×1,000 and enlarged onto the computer video monitor with Flashpoint VQA 3.4 software (Integral Technologies; Indianapolis, Indiana), resulting in a final magnification of ×8,000. Myocytes were measured for myocyte diameter and cross-sectional area, using Image Pro Plus software (Media Cybernetics; Silver Spring, MD).

Western blot for Bcl-2 and Bax protein expression. Protein was extracted from frozen left ventricular tissue. Protein concentration was determined by using a bicinchoninic acid protein assay reagent (Pierce; Rockford, IL) and bovine serum albumin as a standard. Aliquots containing 40 μg of protein was loaded on 12% SDS-PAGE. Equal loading proteins were confirmed by Coomassie blue staining. Proteins were transferred to a polyvinylidene difluoride membrane. Mouse anti-Bcl-2 monoclonal antibody (Santa Cruz Biotechnology; Santa Cruz, CA) and mouse anti-Bax monoclonal antibody (Santa Cruz Biotechnology) were used for Bcl-2 and Bax detection, respectively. The Phototope-HP R Western blot detection kit (New England Biolab; Beverly, MA) was used to visualize the bands. The bands were scanned by a GS-700 imaging densitometer (Bio-Rad; Hercules, CA).

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CA) and quantified using Quantity One program (Bio-Rad). The optical density of tissue samples was normalized to a control sample in arbitrary densitometry unit.

The persons counting apoptotic cardiac myocytes, measuring myocyte size, and performing Western blot analyses for Bcl-2 and Bax proteins were blinded to the drug assignment in the study.

Statistical analysis. Results are presented as means ± SE. Data were analyzed with RS/1 Research System (Bolt, Beranek, and Newman Software Products; Cambridge, MA). Analysis of variance and post hoc Bonferroni simultaneous confidence intervals for all comparisons were used to determine the statistical significance of differences among the four CHF groups and sham animals. A probability value of <0.05 was considered significant.

To study the correlation of total myocyte cellular oxidative stress with either left ventricular function or myocyte apoptosis, we performed linear correlation analysis of cardiac GSH/GSSG with the changes of left ventricular EDD and FS and the number of apoptotic myocytes.

RESULTS

Clinical manifestation of CHF and resting hemodynamics. Table 1 shows body weights, heart weights, and resting hemodynamics of all experimental animals with CHF or sham operation at the end of 8-wk treatment. There were no differences in any of parameters among the four sham groups. Rapid cardiac pacing increased heart weight, left ventricular end-diastolic pressure, and plasma NE, and decreased left ventricular FS and the number of apoptotic myocytes.

Table 1. Body and heart weights, resting hemodynamics, and plasma norepinephrine in sham and CHF animals

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Carvedilol</th>
<th>Metoprolol</th>
<th>Prop + Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>LVW/body wt, g/kg</td>
<td>1.04 ± 0.04</td>
<td>1.08 ± 0.04</td>
<td>1.05 ± 0.05</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>255 ± 8</td>
<td>278 ± 10</td>
<td>261 ± 4</td>
<td>239 ± 14</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>87 ± 4</td>
<td>94 ± 5</td>
<td>84 ± 2</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>LV dP/dt, mmHg/s</td>
<td>3291 ± 237</td>
<td>3737 ± 259</td>
<td>2996 ± 230</td>
<td>2797 ± 156</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8 ± 1</td>
<td>1 ± 1</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Plasma [NE], ng/ml</td>
<td>0.14 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.19 ± 0.02</td>
</tr>
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</table>

Values are means ± SE. Congestive heart failure (CHF) animals exhibited increased left ventricular (LV) weight (LVW), LV end-diastolic pressure (LVEDP), and plasma norepinephrine (NE) concentration and reduced first derivative of LV pressure (LV dP/dt). These changes were not significantly altered by treatment with carvedilol, metoprolol, or propranolol plus doxazosin (Prop + Dox). MABP, mean aortic blood pressure. *P < 0.05 compared with the placebo group (sham), as measured by ANOVA and Bonferroni simultaneous confidence intervals for all comparisons.

Isoproterenol-induced responses did not differ significantly among the three drug-treated groups. The increase in left ventricular EDD and decrease in left ventricular FS decreased by 14%. Figure 1 also shows that in the CHF animals. The average changes during the last 3 wk of pacing are shown in Fig. 1. Figure 1 shows that in the placebo-treated CHF animals, the left ventricular EDD increased by 2.8 ± 0.2 mm or 20% of the baseline dimension, and left ventricular FS decreased by 14%. Figure 1 also shows that the increase in left ventricular EDD and decrease in left ventricular FS were stable during the 8 wk of study in sham animals regardless of the treatment they received.

Intergroup comparisons were determined by Bonferroni simultaneous confidence intervals for all comparisons, showing a significant reduction (*P < 0.05) of the isoproterenol-induced increases of HR and LV dP/dt responses by treatment with carvedilol, metoprolol, or Prop + Dox compared with placebo-treated animals. Isoproterenol-induced increases of HR and LV dP/dt responses did not differ significantly among the 3 drug-treated groups.

Echocardiographic changes in the development of heart failure. Left ventricular EDD and FS were stable during the 8 wk of study in sham animals regardless of the treatment they received. Data from the four sham groups were pooled and presented in Fig. 1. In contrast, rapid ventricular pacing increased left ventricular EDD and reduced left ventricular FS in CHF animals. The average changes during the last 3 wk of pacing are shown in Fig. 1. Figure 1 shows that in the placebo-treated CHF animals, the left ventricular EDD increased by 2.8 ± 0.2 mm or 20% of the baseline dimension, and left ventricular FS decreased by 14%. Figure 1 also shows that the increase in left ventricular EDD and decrease in left ventricular FS were stable during the 8 wk of study in sham animals regardless of the treatment they received.

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ventricular FS were reduced by both carvedilol and metoprolol, but the changes produced by propranolol plus doxazosin were relatively small and statistically insignificant compared with the placebo-treated CHF group.

Myocardial GSH and mtDNA 8-oxo-dG. Figure 2 shows the myocardial tissue GSH/GSSG and mtDNA 8-oxo-dG/dG ratio in the sham and CHF animals. The drug regimens did not change the oxidative stress indices in sham animals, and the results from four sham groups were pooled and shown in the figure. In contrast, rapid cardiac pacing decreased GSH/GSSG and increased the ratio of mtDNA 8-oxo-dG to dG compared with sham animals. Increase in mtDNA 8-oxo-dG in CHF animals was caused by a 72% increase in 8-oxo-dG (15.6 ± 2.3 vs. 9.1 ± 0.5 pg/g), and a 25% decrease in mtDNA dG (0.14 ± 0.01 vs. 0.19 ± 0.01 μg/g). Figure 2 also shows that β-receptor blocker treatment reduced the decrease of tissue GSH/GSSG and increase of mtDNA 8-oxo-dG/dG ratio, but the changes were most marked in the carvedilol-treated CHF animals and least in the propranolol plus doxazosin-treated animals.

Myocyte apoptosis. Figure 3A shows the positive control of immunohistochemical staining of single-stranded DNA of rabbit ventricular muscle tissue section after proteinase K treatment. Representative pictures from a sham, a CHF placebo, and a CHF carvedilol animal are shown in Fig. 3, B–D, respectively.

Administrations of β-receptor blockers produced no changes in the number of apoptotic myocytes in sham animals (8.5 ± 0.8, 8.6 ± 0.8, and 8.9 ± 1.1 apoptotic nuclei/10,000 cardiomyocytes in the carvedilol-, metoprolol-, and propranolol plus doxazosin-treated animals compared with 8.1 ± 0.8 apoptotic nuclei/10,000 myocytes in placebo-treated sham animals). Data from the four sham groups were pooled and shown as a single group in Fig. 4. In contrast, rapid cardiac pacing increased the number of apoptotic nuclei in the left ventricular myocardium. Administration of carvedilol abolished the increase of apoptotic nuclei in CHF animals. Myocyte apoptosis in CHF was also reduced by metoprolol and propranolol plus doxazosin, but the changes produced by propranolol plus doxazosin were smaller than that produced by carvedilol treatment.

Cardiac myocyte size. Figure 5 shows that cardiac myocytes were increased in both cross-sectional area and cell diameter in the CHF animals. There was a 75% increase in myocyte cross-sectional area in the placebo-treated CHF animals compared with the sham animal. This increase in cell cross-sectional area produced by rapid ventricular pacing was reduced by 41% and 51% in the carvedilol- and metoprolol-treated animals, respectively. propranolol plus doxazosin were smaller than that produced by carvedilol treatment.
duced by carvedilol and metoprolol treatment. In contrast, myocyte diameter increased only 25% in CHF, and the reduction in cell diameter by the β-receptor blockers was not statistically significant.

Proapoptotic Bax and antiapoptotic Bcl-2 protein expression. Figure 6 shows changes of myocardial Bcl-2, Bax, and Bcl-2-to-Bax ratio (Bcl-2/Bax) in sham and CHF animals. The figure shows that compared with the sham animals, Bcl-2 protein expression was decreased in the CHF animals (0.75 ± 0.02 vs. 1.00 ± 0.02 arbitrary units). This was associated with an increase in Bax protein (1.25 ± 0.04 vs. 1.03 ± 0.02 arbitrary units) and the resultant decrease of Bcl-2/Bax (0.59 ± 0.03 vs. 0.98 ± 0.03) in CHF animals. Drug treatments had no effects in either Bcl-2 or Bax content in sham animals. Carvedilol and metoprolol prevented the decrease in Bcl-2 protein in the CHF animals; propranolol plus doxazosin treatment resulted in only partial restoration of myocardial Bcl-2 of the failing myocardium. Carvedilol treatment also prevented increase in Bax and decrease in Bcl-2/Bax in CHF animals, but because Bax did not change significantly in the CHF animals treated with metoprolol or propranolol plus doxazosin, the decrease of Bcl-2/Bax was only partially prevented in the latter two groups of animals.

Correlation of GSH/GSSG with echocardiographic data and apoptotic myocyte index. Figure 7 shows the changes of left ventricular EDD and left ventricular FS as a function of left ventricular volume. ANOVA showed significance of differences among the groups (F = 31.94; df = 4, 72; P < 0.001). Statistical significance of differences between the groups was determined by Bonferroni simultaneous confidence intervals. Findings indicate that the number of apoptotic cells was increased in CHF and that this change was reduced by Carv, Meto, and Prop + Dox. Also, the effect of Prop + Dox appeared to be less than that of Carv in the CHF animals. *P < 0.05 compared with sham animals; †P < 0.05 compared with CHF Placebo; ‡P < 0.05 compared with CHF Carv.

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Fig. 3. Increased myocyte apoptosis in rapid cardiac pacing-induced CHF. Apoptotic myocytes were defined by nuclei (indicated by arrows) positively stained by anti-single-stranded DNA monoclonal antibody. A: positive control showing many positively stained nuclei (arrows, 3 representative nuclei) as induced by proteinase K. C: apoptotic myocyte in an untreated CHF animal (CHF placebo). No apoptotic myocytes were seen in a sham animal (B) or a CHF animal treated with Carv (D).

Fig. 4. Effects of rapid cardiac pacing-induced CHF and drug interventions on the number of apoptotic myocytes measured by anti-single-stranded DNA monoclonal antibody-stained nuclei in the LV myocardium. Results were obtained from sham (n = 35) and CHF animals treated with placebo (n = 15), Carv (n = 11), Meto (n = 8), and Prop + Dox (n = 8). Bars denote SE. ANOVA showed significance of differences among the groups (F = 31.94; df = 4, 72; P < 0.001). Statistical significance of differences between the groups was determined by Bonferroni simultaneous confidence intervals. Findings indicate that the number of apoptotic cells was increased in CHF and that this change was reduced by Carv, Meto, and Prop + Dox. Also, the effect of Prop + Dox appeared to be less than that of Carv in the CHF animals. *P < 0.05 compared with sham animals; †P < 0.05 compared with CHF Placebo; ‡P < 0.05 compared with CHF Carv.
A 

B 

SHAM Placebo Carv Meto 

Fig. 5. Effects of rapid cardiac pacing-induced CHF and drug interventions on cardiomyocyte diameter and cross-sectional area. Results were obtained from sham (n = 6) and CHF animals treated with placebo (n = 8), Carv (n = 6), and Meto (n = 6). ANOVA showed significance of differences exists in cell cross-sectional area (F = 11.74; df = 3, 22; P < 0.001), and cell diameter (F = 3.84; df = 3, 22; P = 0.024) among the groups. Statistical significance of differences between the groups was determined by Bonferroni simultaneous confidence intervals. Findings indicate that myocyte size was increased in CHF animals and that treatment with Carv and Meto reduced the increase in myocyte size produced by rapid cardiac pacing. *P < 0.05 compared with sham animals; †P < 0.05 compared with CHF Placebo.

Fig. 6. Effects of rapid cardiac pacing-induced CHF and drug interventions on myocardial Bcl-2, Bax, and Bcl-2/Bax. Left, representative Western blots are shown for Bcl-2 and Bax. Pool data were obtained from sham (n = 41) and CHF animals treated with placebo (n = 15), Carv (n = 9), Meto (n = 9), and Prop + Dox (n = 7). Bars denote SE. ANOVA showed significance of differences between the groups for Bcl-2 (F = 21.81; df = 4, 76; P < 0.001), Bax (F = 44.64; df = 4, 76; P < 0.001) and Bcl-2-to-Bax ratio (F = 50.99; df = 4, 76; P < 0.001). Statistical significance of differences between the groups was determined by Bonferroni simultaneous confidence intervals. The findings indicate that CHF increased Bax protein and decreased Bcl-2 protein. Effects of Prop + Dox were the least among the 3 drug interventions. *P < 0.05 compared with sham animals; †P < 0.05 compared with CHF Placebo; ‡P < 0.05 compared with CHF Carv.

DISCUSSION

The present study shows that carvedilol reduced total cellular oxidative stress and myocyte apoptosis and cell hypertrophy in CHF and that these effects probably contributed to the attenuation of left ventricular remodeling in CHF. Qualitatively similar effects were produced by metoprolol. In contrast, propranolol plus doxazosin did not significantly affect the state of oxidative stress and exerted a smaller protective effect on myocyte apoptosis and progression of ventricular remodeling in CHF. The results suggest that antioxidant property of carvedilol and metoprolol is desirable and mechanistically important for the amelioration of cardiac remodeling and myocyte apoptosis in CHF.

Doses of carvedilol, metoprolol, and propranolol plus doxazosin were chosen empirically on the basis of relative potencies of the three β-receptor blockers. Efficacies of the medications as β-receptor blockers were evidenced by slowing of heart rate to a similar extent in the drug-treated sham animals. The three β-receptor blocker regimens also produced quantitatively similar reductions of the isoproterenol-induced increases of heart rate and left ventricular dP/dt in the sham-operated animals (Table 2). Findings indicate that the doses employed, carvedilol, metoprolol, and propranolol plus doxazosin produced similar degree of β-receptor inhibition in the animals.

Evidence has accumulated that oxidative stress is increased in experimental and human heart failure (43) and that increased oxidative stress may contribute to the progression of heart failure (24). Recent studies have shown that carvedilol acting...
Cardiac hypertrophy, Teiger et al. (44) showed that a wave of myocyte apoptosis occurred during the first 7 days after pressure overload followed by progressive cell growth (hypertrophy) and a decrease in programmed cell death over 30 days. The rate of apoptosis is in the 80–250/10⁵ cardiomyocytes range in the human failing heart (28). However, despite the slow rate of apoptosis, given the short duration (<24 h) required for a myocyte to undergo apoptosis and cleared from the tissue (14), the programmed cell death is considered pathologically important and may contribute a significant loss of myocytes over a long period of time. The importance of cardiac myocyte apoptosis in heart failure was further demonstrated by Wencker et al. (48), using transgenic mice, in that lower levels of myocyte apoptosis (23/10⁵) were induced by conditional overexpression of caspase 8 in the heart.

In our present study, cardiac remodeling was evidenced by the progressive dilation of the left ventricle and decline of left ventricular FS in rapid pacing-induced heart failure animals. This is further supported by increased cardiomyocyte apoptosis and myocyte hypertrophy in the CHF animals. In our study, cell apoptosis occurred at a rate of 8/10⁵ myocytes at baseline in sham rabbits and increased to ~690/10⁵ myocytes in an established heart failure state after 8 wk of rapid cardiac pacing. In dogs, Leri et al. (20) reported using the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) labeling that myocyte apoptosis occurred at a rate of 2/10⁵ myocytes in control myocardium and increased to 400/10⁵ myocytes in the failing heart after 4 wk of rapid cardiac pacing. Quantitative differences between our study and that of Leri et al. (20) are probably related to the differences in animal species, apoptosis detection techniques, and duration of heart failure. Other studies have shown that the apoptotic rates are in the 1/10⁶ to 18/10⁵ range in normal myocardium and in the 50/10⁵ to 350/10⁵ range in the failing heart.

Of note, our study employed a monoclonal antibody against single-stranded DNA that was used to identify apoptotic cells (10, 47). This monoclonal antibody labeling detects early apoptotic cells and is more sensitive than the traditional TUNEL method (12) that detects double-stranded DNA breakage present only during late-stage apoptosis (47). Furthermore, unlike TUNEL staining that also labels necrotic cells, the monoclonal antibody against single-stranded DNA does not detect necrotic cells (11, 47) and is thus more specific for detection of cell apoptosis than the TUNEL assay. In a study utilizing both the TUNEL assay and the monoclonal antibody to single-stranded DNA labeling (34), we found that the number of monoclonal antibody-positive cells were at least twice the amount of TUNEL-positive cells.

Administration of carvedilol and metoprolol attenuated the increase of cardiac myocyte size along with reduction of myocyte apoptosis, in the pacing-induced cardiomyopathic animals. This was associated with the smaller increase of left ventricular diastolic diameter and less decline of left ventricular FS after rapid cardiac pacing in these animals. These animals also exhibited a lower left ventricular end-diastolic pressure during the hemodynamic study at week 8, suggesting an improvement in left ventricular function in vivo. However, the effects of carvedilol and metoprolol on left ventricular end-diastolic pressure were relatively smaller and not statistically significant, given the number of animals studied, and there were no significant differences of peak left ventricular systolic pressure.
dP/dt at rest among the four experimental CHF groups. Discrepancies in the magnitude of changes in cardiac function as measured by the echocardiography and cardiac catheterization have been described before (17, 35). We speculate that the difference between the two states may be related to the different levels of anesthesia used. The deeper level of general anesthesia used for the invasive cardiac catheterization study might have masked the improvement in cardiac hemodynamics produced by carvedilol and metoprolol.

Carvedilol inhibits myocyte apoptosis in myocardial ischemia and reperfusion (52). It also has been shown to protect endothelial cells against apoptosis produced by epinephrine (37) and heart failure (38). Our present study shows that myocyte apoptosis in CHF was reduced by carvedilol and metoprolol. This was associated with a reduction of left ventricular chamber dilation and cardiac myocyte hypertrophy in the treated CHF animals.

Our results further suggest a potential functional linkage between the antiapoptotic and antioxidant effects of carvedilol and metoprolol in CHF. Reduction of myocyte apoptosis also has been shown with antioxidant vitamins in CHF animals (35). However, β-receptor blockade may play an important role in the antiapoptotic effect as well, because propranolol plus doxazosin, which had no major effect on cardiac oxidative stress, produced a reduction of myocyte apoptosis in CHF. The coefficient of determination ($r^2$) for the correlation between GSH/GSSG and the number of apoptotic myocytes suggests the antioxidant effects of β-receptor blockers could only account for 22% of the changes in the number of apoptotic myocytes in CHF. Propranolol has been shown to reduce myocyte apoptosis produced by norepinephrine in cultured myocytes (8).

CHF is associated with a decrease in the antiapoptotic Bcl-2 protein and an increase in proapoptotic Bax protein (20). Our results further demonstrated that carvedilol prevented the changes in both Bcl-2 and Bax proteins, whereas metoprolol reduced the decrease of Bcl-2 but had no effect on Bax in pacing-induced heart failure. Metoprolol also has been shown to increase Bcl-2 protein in CHF produced by intracoronary microembolization (40). Likewise, antioxidant vitamins have been shown to suppress oxidative stress-mediated myocyte apoptosis by the modulation of Bcl-2 and Bax proteins (34). However, the inhibition of β-adrenergic receptors and α1-adrenergic receptors by carvedilol may also be involved in the regulation of Bcl-2 and Bax proteins, because changes in Bcl-2/Bax also have been shown to occur in norepinephrine-treated adult rat ventricular myocytes after atenolol (53) and in spontaneously hypertensive rats after doxazosin (36).

Numerous clinical studies have shown that long-term treatment with β-receptor blockers in patients with heart failure significantly improves cardiac function, lessens symptoms of heart failure (29, 30), and reduces cardiac and total mortality and morbidity (15, 31–33). Experimental studies have further demonstrated that carvedilol decreases myocardial fibrosis and heart weight, improves cardiac function, and increases survival in rats with immune-induced dilated cardiomyopathy (46). Chronic treatment with carvedilol also has been shown to improve ventricular function and reduce cardiomyocyte apoptosis in myocardial turkeys (27). More recently, consistent with our present study, carvedilol was shown to attenuate myocardial inflammation and decrease the severity of myocardiitis in rats by an antioxidant action (50). Also the antiapoptotic effect of carvedilol on cardioprotection may be independent of direct β-adrenergic receptor blockade (41). Because heart rate was kept constant by electric pacing in our CHF animals, attenuation of left ventricular remodeling was independent of slowing of heart rate with β-receptor blocker therapy seen in clinical settings. Conversely, it is possible that persistent rapid ventricular pacing limited the animals to manifest greater reversal of cardiac remodeling or more marked hemodynamic improvement after treatment. Studies should be extended to other experimental heart failure models. Earlier studies have shown that the attenuation by β-receptor blockers of left ventricular remodeling and dysfunction induced by coronary artery stenosis might involve improvement of coronary circulation and reduction of free radicals (49). Nevertheless, in our study, the coefficients of determination ($r^2$) for the correlations between GSH/GSSG and the echocardiographic parameters (Fig. 7) indicate that the antioxidant effects of β-receptor blockers accounted for ~50% of the changes in left ventricular EDD and FS. Thus we conclude that reduction of myocardial oxidative stress by β-receptor blockers plays an important role in the reduction of left ventricular dilation and mechanical dysfunction in CHF. The greater antioxidant effect of carvedilol and metoprolol may also be important in determining the clinical efficacies of β-receptor blocker therapy in the treatment of CHF.

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REFERENCES


