Effects of β-Blocker on Left Ventricular Remodeling in Rats with Volume Overload Cardiac Failure

Masayuki KOBAYASHI¹, Noboru MACHIDA¹, Ryou TANAKA² and Yoshihisa YAMANE²

¹Departments of Veterinary Clinical Oncology and ²Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu, Tokyo 183–8509, Japan

(Received 22 March 2008/Accepted 24 June 2008)

ABSTRACT. The beneficial effects of β-blockers on left ventricular (LV) remodeling have been reported in association with several conditions that cause heart failure, but their effects on the volume overloaded heart failure have not been well defined. Fifty Wistar rats that survived aortocaval (AC) shunt creation were randomly allotted into the following two groups: untreated animals (ACS; n=26) and animals treated with 100 mg/kg/day metoprolol (MP; ACS+MP; n=24). The effects of MP were evaluated at 1, 4 and 12 weeks post-surgery through echocardiographic, hemodynamic and pathologic studies. At 12 weeks post-surgery, LV wall thinning associated with chamber dilatation was observed in ACS but not in ACS+MP. LV end-diastolic pressure and diastolic wall stress were lower in ACS+MP than in ACS. The increase in LV weight was similar in both ACS and ACS+MP at 1 and 4 weeks post-surgery, but at 12 weeks post-surgery, it was significantly greater in ACS+MP than in ACS. At the cellular level, although the cardiac myocyte length progressively increased to a similar extent in both groups, the mean cross-sectional diameter of these cells in ACS+MP was greater than in ACS. In conclusion, MP did not prevent early eccentric hypertrophy in response to volume overload. However, in the late phase of volume overload-induced heart failure, MP appears to allow for myocyte cross-sectional growth and thus prevents LV wall thinning, resulting in a net increase in LV mass. In this manner, MP might contribute to reduction of diastolic wall stress and thereby delay progression of heart failure.

KEY WORDS: aortocaval shunt, β-blocker, ventricular hypertrophy, volume overload.

Cardiac remodeling secondary to volume overload in conditions such as aortic and mitral regurgitation, arteriovenous shunt, anemia and bradycardia is characterized by a marked increase in left ventricular (LV) chamber volume without an increase in wall thickness (eccentric hypertrophy) [4]. Although the remodeling is initially a compensatory response that has the effect of normalizing diastolic wall stress and cardiac output, sustained volume overload may induce progression from adaptive to maladaptive remodeling (LV dilatation with wall thinning) and may ultimately lead to the development of heart failure [4]. Under sustained load conditions, development of cardiac remodeling may be a response not only to hemodynamic load but also to excessive neurohumoral activation (i.e., through renin-angiotensin or the adrenergic system). Excessive or detrimental remodeling independent of the initial hemodynamic load appears to play an important role in transition from the compensatory stage to the heart failure stage [10].

The beneficial effects of β-blockers on cardiac function and survival in patients with heart failure have been clearly demonstrated in a number of clinical trials [14, 19]. It has also been reported in patients with dilated cardiomyopathy that long-term treatment with β-blockers can not only attenuate the progression of LV remodeling but also reverse it [14]. These results suggest that β-blockers have potential beneficial effects on myocardial remodeling in the process of heart failure. However, the effects of β-blockers on hemodynamic volume overload cardiac remodeling have not been defined. The aim of this study was to determine whether β-blockers affect LV remodeling in a rat model of chronic volume overload cardiac hypertrophy and failure.

MATERIALS AND METHODS

Animal model and surgical procedure: All experimental and animal care procedures were approved by the guiding principles of the Tokyo University of Agriculture and Technology and were conducted in accordance with the institution’s guidelines for the care and use of laboratory animals. Seventy, 7-week-old, male Wistar rats (Saitama Experimental Animal Laboratory, Saitama, Japan) with body weights of 190 to 220 g were used in the present study. Operative procedures were carried out under full surgical anesthesia with intraperitoneal administration of sodium pentobarbital (50 mg/kg). LV volume overload was induced according to the procedures described by Garcia and Diebold [11]. Briefly, the abdominal aorta and vena cava were exposed through a midline abdominal incision. An 18-gauge disposable needle was inserted into the exposed abdominal aorta and advanced into the vena cava to create an aortocaval (AC) shunt. The needle was withdrawn, and the ventral aortic puncture point was sealed with cyanoacrylate glue. The patency of the shunt was verified visually on the basis of the swelling of the vena cava and mixing of arterial blood with venous blood. The abdominal muscle and skin incisions were closed by standard techniques with absorbable...
suture. AC shunt surgery was performed on 55 rats (‘AC shunt rats’). Fifteen sham control rats underwent the same surgery but without hemodynamic intervention. The rats were maintained 5 per cage in a temperature-controlled room with a 12-hr light-dark cycle and were fed a standard laboratory rat diet.

Experimental protocol: Twenty-four hours after AC shunt surgery, the surviving rats were randomly allotted to either an untreated group or a group treated with a $\beta_1$-selective blocker, metoprolol (MP; Sigma, St. Louis, U.S.A.). MP was dissolved in drinking water at a dose of 100 mg/kg/day on the basis of preliminary data concerning the volume of water consumed per day. One week after surgery, an echocardiographic study was performed on all the surviving rats, and within 24 hr after the echocardiographic study, 5 rats randomly selected from each group were used for both hemodynamic and pathological studies. The same studies were also performed 4 weeks after surgery. Twelve weeks after surgery, all the remaining rats were used for echocardiographic, hemodynamic and pathological studies.

Echocardiographic study: Transthoracic echocardiography was performed using an ultrasound system (SSD-5500, Aloka, Tokyo, Japan) with a 10-MHz transducer. The rats were sedated with intraperitoneal ketamine HCl (50 mg/kg) and xylazine (10 mg/kg). Standard echocardiography techniques were used to obtain M-mode echocardiograms from short-axis views at the papillary muscle level of the LV. The thickness of the interventricular septum and LV posterior wall and the internal diameter of the LV at end-diastole (IVSd, LVPWd and LVIDd, respectively) and at end-systole (IVSs, LVPWs and LVIDs, respectively) were measured in at least three consecutive cardiac cycles by the American Society for Echocardiography leading edge method [17]. LV relative wall thickness was calculated as 2 × (LVIDs/LVPWs) [1+(LVPWs/LVIDs)] and diastolic wall stress=0.334 × LVEDP × (LVIDd/LVPWd) [1+(LVPWd/LVIDd)].

Pathologic study: After the hemodynamic study, the heart was arrested in diastole by intraventricular injection of KCl (1 mmol/l). The arrested heart was removed by cross-clamping and cutting of the great vessels at the heart base. The attached great vessel roots and pericardium were dissected free, and the atria were removed at the coronary sulcus. The free right ventricular (RV) wall was dissected from the combined left ventricle and septum by cutting along the junction with the interventricular septum. The cardiac portions were blotted dry and weighed. The LV weight was defined as the weight of the LV free wall plus interventricular septum. The combined LV and septum were then cut across the minor axis approximately one-third the distance from the base to the apex. Both lungs were also extirpated from the thoracic cavity and weighed. The heart tissues were immersion-fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (HE). The heart sections were also stained with picric acid-sirius red and Masson’s trichrome.

To determine the degree of myocardial fibrosis, cross-sections of the LV stained with picric acid-sirius red were divided into endomyocardial and epicardial halves, and a minimum of 50 fields were measured from both the inner and outer LV halves under a 10x objective. The tissues were analyzed with a video imaging system (MacSCOPE ver.2.6, Mitani Corp., Tokyo, Japan), and the mean percentage volume of connective tissue for each animal was calculated from the total areas measured; these percentage values were combined for determination of group means.

Myocyte cross-sectional diameter (CSD; n=100 in each animal) was measured by video microscopy (BX50, Olympus, Tokyo, Japan) in sections stained with HE, and suitable cross-sections were defined as having nearly rounded and nucleated myocytes. Isolated myocytes were prepared by the KOH method described previously [13]. Briefly, formalin-fixed myocardial tissues from the LV free wall were cut into approximately 1 × 1 mm pieces and placed into 12.5 mol/l KOH solution. The samples were shaken gently at room temperature for approximately 20 hr at slow speed. They were then placed into 0.1-mol/l phosphate-buffered saline solution. The samples were vortexed vigorously for approximately 3 min and filtered through 250-µm nylon mesh, and the myocytes were suspended in 10% phosphate-buffered formalin. Myocyte length (n=100 for each animal) was measured by light microscopy of the isolated cells settled on a glass slide and examined with a video imaging system.

Statistical analysis: All data are presented as means ± S.E. computed from the average measurements obtained from each group. Statistical evaluation was performed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test for group differences at each time point. P<0.05 was considered to be statistically significant.
 RESULTS

Mortality: The mortality rate within 24 hr after AC shunt surgery was 9.1% (5 of the 55 rats that underwent surgery). The animals that survived for 24 hr after surgery (50 rats) were randomly allotted to an untreated group (n=26) and a group treated with MP (n=24). Seven (26.9%) of the untreated AC shunt rats and 5 (20.8%) of the MP-treated AC shunt rats died by 1 week post-surgery. During the second week, natural death occurred in one of the untreated AC shunt rats and in two of the MP-treated AC shunt rats; subsequently, no more rats died. Postmortem examination revealed signs of acute or subacute congestive heart failure, including cardiomegaly, pulmonary congestion and edema and pleural effusion. There were no significant differences in mortality rates at any time point between the untreated and MP-treated AC shunt rats. Sham control rats showed no apparent signs of congestive heart failure, and no deaths were observed throughout the study in this group.

Echocardiographic study: Representative M-mode echocardiograms in each group at 12 weeks post-surgery are shown in Fig. 1. LVIDd and LVIDs progressively increased in both the untreated and MP-treated AC shunt rats and were larger in both these groups than in the sham controls at 1, 4 and 12 weeks post-surgery, but there was no significant difference between the two AC shunt rat groups (Table 1). There were no significant differences in LVPWd and IVSd among the groups at 1 and 4 weeks post-surgery, but at 12 weeks post-surgery, these parameters were smaller in the untreated AC shunt rats than in the MP-treated AC shunt rats and sham controls, and there were significant differences between the two groups of AC shunt rats (Table 1). LV relative wall thickness at 12 weeks post-surgery was significantly smaller in the both untreated and MP-treated AC shunt rats than in the sham rats, and the value in the untreated rats was much smaller than that in the MP-treated rats (Fig. 2A). LVFS was almost constant and remained at a normal value in all groups throughout the study (Table 1).

Hemodynamic study: There were no significant differences in heart rates between the sham control and untreated AC shunt rats at any time point (Table 2). However, the mean heart rate of the MP-treated AC shunt rats was lower than that of the untreated AC shunt rats and sham control rats at each time point, and there were significant differences at 4 and 12 weeks post-surgery (Table 2). The systolic blood pressures of both the untreated and MP-treated AC shunt rats were lower than in the sham control rats at most time points, but there was no significant difference between the two groups of AC shunt rats (Table 2). LVESP was low in both the untreated and MP-treated AC shunt rats compared with the sham control rats, and LVESP was significantly lower in the untreated AC shunt rats than in the sham control rats at 1 week post-surgery (Table 2). LVEDP was

---

Table 1. Echocardiographic data of sham, untreated and metoprolol (MP)-treated rats after aortocaval shunt surgery

<table>
<thead>
<tr>
<th></th>
<th>LVIDd (mm)</th>
<th>LVIDs (mm)</th>
<th>LVPWd (mm)</th>
<th>IVSd (mm)</th>
<th>LVFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=15)</td>
<td>6.4 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>34.6 ± 2.2</td>
</tr>
<tr>
<td>Untreated shunt (n=19)</td>
<td>8.0 ± 0.1</td>
<td>4.9 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>38.0 ± 1.7</td>
</tr>
<tr>
<td>MP-treated shunt (n=19)</td>
<td>8.1 ± 0.2</td>
<td>5.3 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>34.1 ± 1.1</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=10)</td>
<td>6.7 ± 0.3</td>
<td>4.2 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>35.2 ± 1.8</td>
</tr>
<tr>
<td>Untreated shunt (n=13)</td>
<td>9.0 ± 0.3</td>
<td>6.0 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>36.9 ± 0.5</td>
</tr>
<tr>
<td>MP-treated shunt (n=12)</td>
<td>9.2 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>34.9 ± 1.7</td>
</tr>
<tr>
<td>12 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=5)</td>
<td>7.4 ± 0.3</td>
<td>4.4 ± 0.5</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>35.8 ± 2.2</td>
</tr>
<tr>
<td>Untreated shunt (n=8)</td>
<td>10.6 ± 0.4</td>
<td>7.0 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>34.1 ± 1.2</td>
</tr>
<tr>
<td>MP-treated shunt (n=7)</td>
<td>10.9 ± 0.2</td>
<td>7.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>31.1 ± 2.0</td>
</tr>
</tbody>
</table>

Data are shown as means ± S.E. LVIDd, left ventricular internal diameter at diastole; LVIDs, left ventricular internal diameter at systole; LVPWd, left ventricular posterior wall thickness at diastole; IVSd, interventricular septum thickness at diastole; LVFS, left ventricular fractional shortening. a) P<0.05, b) P<0.01 vs sham. c) P<0.05, d) P<0.01 vs untreated shunt.
significantly higher in the untreated AC shunt rats than in the sham control rats at each time point, but the values remained almost constant after 1 week post-surgery (Table 2). Although there were no significant differences in LVEDP between the untreated and MP-treated AC shunt rats at each time point post-surgery, LVEDP was much higher in the untreated shunt rats than in the MP-treated shunt rats at 12 weeks post-surgery (Table 2). Systolic wall stress was similar among the three groups, although the value in the untreated AC shunt rats at 1 week post-surgery was significantly lower than that in the sham control rats (Table 2). Diastolic wall stress progressively increased in both the untreated and MP-treated AC shunt rats and was larger than in the sham control rats at 4 and 12 weeks post-surgery (Table 2). Diastolic wall stress was significantly lower in the MP-treated shunt rats than in the untreated shunt rats at 12 weeks post-surgery (Table 2).

Pathologic study: There was no difference in body weight among the three groups at any time point (Table 3). On pathologic examination at 12 weeks post-surgery, apparent signs of congestive heart failure (subcutaneous edema, pleural effusion and pulmonary congestion and edema) were
DISCUSSION

We examined structural changes in the LV chamber and myocytes and LV function in a rat model of volume overload over 12 weeks after AC shunt creation. Our observations of untreated AC shunt rats were almost the same as those of previous studies using the same rat model [3, 12, 21]. The course of volume overload cardiac hypertrophy consisted of three phases: an early phase (generation of the disease), intermediate phase (compensation of the disease) and late phase (decompensation of the disease). During the first week post-surgery, more than 20% of the untreated and MP-treated AC shunt rats died of congestive heart failure. The rats that died at this early phase were presumably unable to compensate for the abrupt increase in preload. The next phase (at about 4 weeks post-surgery) seems to correspond with the phase of compensation for the sustained increase in preload. At this phase, eccentric hypertrophy was established in most of the AC shunt rats; this was characterized by a progressive increase in LV internal diameter and preservation of wall thickness. Changes in myocyte shape were characterized by both longitudinal and cross-sectional growth of myocytes. In this phase, the remodeling might have still been compensating for the elevation of LVEDP and diastolic wall stress because mortality was dramatically reduced compared with the early phase (the first week post-surgery). At 12 weeks post-surgery, a further increase in the LV internal diameter together with wall thinning resulted in a more dilated LV. Further elongation of myocytes without cross-sectional growth occurred in this phase. The LV dilatation was accompanied by signs of cardiac decompensation, such as significant increases in LVEDP, diastolic wall stress and lung weight. Although no apparent symptoms of heart failure or death were observed in the untreated AC shunt rats in this phase, the hemodynamic and pathologic findings in these rats indicated progression to a decompensatory stage.

The effects of MP on volume overload cardiac hypertrophy and failure caused by AC shunt are reported here for the first time. The major findings are as follows: (1) MP had no effect on the development of eccentric hypertrophy during the early phase (the first 4 weeks after surgery); (2) MP pre-
vented LV wall thinning and promoted a larger hypertrophic response in later phases (12 weeks after surgery); (3) MP did not influence myocyte elongation but promoted myocyte cross-sectional growth at the cellular level in later phases; and (4) MP attenuated the increase in LVEDP and diastolic wall stress and pulmonary congestion. The myocyte cross-sectional growth in the MP-treated AC shunt rats at 12 weeks post-surgery may have been mainly responsible for the preservation of LV wall thickness and the further increase in LV weight because no histological evidence of increases in interstitial components was detected. It is likely that the cross-sectional myocyte growth is due to the direct effects of MP because the systolic load conditions (as shown by LVESP and systolic wall stress) did not differ between the untreated and MP-treated AC shunt rats. The similar hypertrophic change in the RV weight may also suggest the contribution of factors independent of hemodynamic load.

Unfortunately, the precise mechanisms of the hypertrophic effects of MP on myocytes in the volume overloaded heart were not clarified in the present study. Tsutsui et al. demonstrated that a six month-treatment of β-blocker promotes myocyte hypertrophy and myofibril density in dogs with a volume overload induced by experimental mitral regurgitation [20]. They suspected that a direct effect of β-blocker in protecting myocytes from catecholamine toxicity is one possible mechanism by which this drug enables the heart to maintain adaptive eccentric hypertrophy in volume overload [20]. Excessive activation of the sympathetic nervous system plays a major role in the development of detrimental LV remodeling and dilatation [1], and catecholamines reduce viability and protein synthesis in isolated adult cardiac myocytes [15]. However, Commul et al. showed that there was no increase in the plasma concentrations of catecholamines and a decrease in the ventricular catecholamine level in the same AC shunt rats at 4 weeks post-surgery [7]. Wang et al. demonstrated the unique characteristics of volume overloaded hearts in AC shunt rats: the β-receptor density (upregulation) and adenylyl cyclase activity increased significantly, even in the heart failure stage (16 weeks post-surgery) [21]. These findings indicate that myocytes in AC shunt rats may not be exposed to excessive amounts of catecholamines even in the later stages of heart failure. Thus, the effects of MP in allowing adequate hypertrophy might be due to another mechanism unrelated to catecholamine toxicity.

One possible mechanism is the effects of β-blocker in preventing myocardial interstitial changes in the volume overloaded heart. Recent studies have demonstrated that changes in interstitial matrix components play an important role in LV chamber dilatation [5, 8]. Degeneration of collagen fibers and lysis of the collagen framework are most likely responsible for the myocyte side-to-side slippage that leads to chamber dilatation and wall thinning [5, 8]. We believe that this slippage mechanism contributed to ventricular dilatation in the AC shunt rat, because there was no accompanying increase in wall thickness in any phase, despite the myocyte cross-sectional growth. Senzaki et al. recently demonstrated that β-blocker prevents activation of matrix metalloproteinases (MMPs) in dogs with cardiac dysfunction induced by rapid-pacing and angiotensin II infusion and suggested that β-blocker may limit extracellular matrix changes [18]. We speculate that MP-dependent prevention of extracellular matrix degradation might be involved in the hypertrophic effect of MP, although no significant change in myocardial collagen content was observed in the untreated and MP-treated AC shunt rats. Myocytes without the protection of the normal collagen framework might be easily influenced by mechanical forces, which might lead the inadequate myocyte growth. Further biochemical evaluations of the quantity and quality of collagen (i.e., collagen concentration, ratio of collagen type I to type III and collagen cross-linking) and MMPs activity are required.

Reduction of heart rate by β-blockers may lead to improvement of diastolic function through lengthening of diastole [6]. Furthermore, β-blocker-induced bradycardia may be beneficial in protecting myocytes through improvement of the relationship between myocardial blood flow and oxygen consumption [16]. Improvement of bioenergetics of myocytes by decreasing the heart rate might contribute to adaptive myocyte growth. Clarification of the effects of β-blocker on chamber dilatation and wall remodeling requires further investigation.

Further studies are also needed to determine whether the effects of MP on LV remodeling prevent end-stage cardiac dysfunction and improve survival in volume overload heart failure. We did not perform any evaluations beyond 12 weeks post-surgery. In the same model, Brower and Janicki observed overt heart failure at about 20 weeks after surgery, by which time more than 80% of the rats had died [2]. They clearly showed that intrinsic myocyte contractility decreased in AC shunt rats. In our study, apparent signs of heart failure were observed in only 37.6% of the untreated AC shunt rats at 12 weeks after surgery. Furthermore, the LV dilatation at that stage was not associated with systolic chamber dysfunction (i.e., normal LVFS). Thus, our AC shunt rats did not reach end-stage heart failure, which is when systolic dysfunction becomes evident.

In conclusion, MP did not prevent early eccentric hypertrophy in response to volume overload. However, in the late phase of volume overload-induced heart failure, MP appears to allow for myocyte cross-sectional growth and thus prevents LV wall thinning, resulting in a net increase in LV mass in treated animals. In this manner, MP might contribute to reduce diastolic wall stress and thereby delay progression of heart failure.

REFERENCES

EFFECTS OF \(\beta\)-BLOCKER ON THE VOLUME OVERLOADED HEART