Usefulness of Carvedilol in the Treatment of Chronic Aortic Valve Regurgitation

Adnane Zendaoui, PharmD*; Dominic Lachance, PhD*; Élise Roussel, MSc; Jacques Couet, PhD; Marie Arsenault, MD

Background—Aortic regurgitation (AR) is a chronic disease for which there is currently no approved medical treatment. We previously reported in an animal model that β-blockade with metoprolol exerted beneficial effects on left ventricular remodeling and survival. Despite the recent publication of promising human data, β-blockade in chronic AR remains controversial. More data are needed to support this potentially new treatment strategy. We hypothesized that carvedilol might be another safe treatment option in chronic AR, considering its combined β-blocking and α-blocking effects and proven efficacy in patients with established heart failure.

Methods and Results—The effects of a 6-month treatment with carvedilol 30 mg/kg/d orally were evaluated in adult Wistar rats with severe AR. Sham-operated and untreated AR animals were used as controls. Carvedilol treatment resulted in less left ventricular hypertrophy and dilatation. Ejection fraction was improved and filling pressures were reduced by carvedilol. β1-Receptor expression was also improved as well as myocardial capillary density. Those beneficial effects were noted despite the presence of drug-induced bradycardia.

Conclusions—Carvedilol exerted protective effects against volume-overload cardiomyopathy in this model of aortic valve regurgitation with preserved ejection fraction. These results suggest a protective class effect of β-blockers. Combined with the recent publication of promising human data, our findings support the need to carefully design a prospective study in humans to evaluate the effects of β-blockers in chronic aortic valve regurgitation. (Circ Heart Fail. 2011;4:207-213.)

Key Words: aortic valve regurgitation • volume overload • left ventricular hypertrophy • β-blockers

Chronic aortic valve regurgitation (AR) is a disease for which there is no approved pharmacological treatment. Chronic AR will slowly alter the left ventricle (LV) over decades, causing severe dilatation, eccentric hypertrophy, and eventually diastolic and systolic heart failure. The treatment of patients with chronic AR is currently limited to surgical aortic valve replacement when specific clinical or echocardiographic criteria are reached.1

Clinical Perspective on p 213

Many small clinical trials have been designed over the past decades to search for an effective pharmacological treatment for AR, but these trials have unfortunately been inconclusive or contradictory, probably because of methodological differences or small sample size.2-4 Based on the hypothesis that the adrenergic system is overactivated early in the course of chronic AR even before heart failure occurs, we have previously evaluated the effects of β-blockade with metoprolol in an animal model of chronic AR and found that it improved LV function, reduced LV hypertrophy, and increased survival.5,6 Recently, Sampat et al7 reported beneficial effects of β-blockers in a retrospective study of 756 patients. The use of β-blockers in chronic AR nevertheless remains controversial, mostly because drug-induced bradycardia may prolong diastole and consequently augment regurgitant time and volume. More data are needed to strengthen the hypothesis that β-blockade early in the course of chronic AR may be protective. We therefore designed the present study to evaluate another β-blocking agent: carvedilol. Considering its β-blocking and α-blocking properties, we hypothesized that it would improve hemodynamics and LV remodeling in rats with chronic severe AR and preserved ejection fraction.

Methods

Animal Model of AR

Twenty-four male Wistar rats (weight, 300 to 350 g; Charles River, Quebec, Canada) had severe AR induced by retrograde puncture of the aortic valve leaflets as previously described8,9 and were randomly
Table 1. Data at Time of Euthanasia

<table>
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<tr>
<th>Parameters</th>
<th>Sham (10/10)</th>
<th>Sham Carv (10/10)</th>
<th>AR (8/12)</th>
<th>AR Carv (9/12)</th>
<th>Disease</th>
<th>Treatment</th>
<th>D×T</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>766±10.3</td>
<td>771±17.7</td>
<td>740±11.7</td>
<td>758±28.0</td>
<td>0.33</td>
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<td>Tibial length, mm</td>
<td>65.8±0.30</td>
<td>65.6±0.47</td>
<td>64.4±0.51</td>
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<td>0.29</td>
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<td>Heart weight, mg</td>
<td>1476±20.5</td>
<td>1508±45.6</td>
<td>2661±107.3</td>
<td>2190±91.3†</td>
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<td>0.006</td>
<td>0.0009</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>1023±19.7</td>
<td>1028±31.8</td>
<td>1881±72.4</td>
<td>1598±59.8*</td>
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<td>RV weight, mg</td>
<td>301±5.7</td>
<td>344±3.6</td>
<td>459±23.5</td>
<td>369±19.2*</td>
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<td>0.009</td>
<td>0.039</td>
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<td>Left atria weight, mg</td>
<td>33±2.4</td>
<td>39±4.3</td>
<td>93±10.6</td>
<td>56.5±4.3*</td>
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<td>0.034</td>
<td>0.0037</td>
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<td>Lung weight, g</td>
<td>2.6±0.14</td>
<td>2.5±0.16</td>
<td>3.4±0.31</td>
<td>2.9±0.25</td>
<td>0.046</td>
<td>0.36</td>
<td>0.43</td>
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</table>

Carv indicates carvedilol; RV, right ventricle. Values are expressed as mean±SEM. P values from 2-way ANOVA analysis are shown on the right to evaluate separately the general impact of disease or treatment. If interaction between disease and treatment (D×T) was found to have a P value below 0.05, a Bonferroni post-test was conducted: *P<0.01 and †P<0.001 versus untreated AR group.

Analysis of mRNA Accumulation by Quantitative Reverse Transcriptase–Polymerase Chain Reaction

Tissues stored frozen in RNAlater (Ambion, Austin, TX) were homogenized in Trizol (Invitrogen, Burlington, Ontario, Canada), and quantitative reverse transcriptase–polymerase chain reaction was conducted on the appropriate tissue samples. QuantiTech Primers (Qiagen, Mississauga, Ontario, Canada) used for this study are listed in the online-only Data Supplement Table S1. Cyclophilin A was used as a control. The quantification of gene expression was based on the 2ΔΔCt method. Results are expressed relative to the sham group mRNA levels, which were arbitrarily fixed at 1. Natriuretic peptide type A (ANP) and type B (BNP) expressions were evaluated considering their close relation to filling pressures and symptomatic heart failure. Procollagens 1 and 3 as well as fibronectin expressions were studied as key components of interstitial myocardial fibrosis. The expression of myocardial adrenoreceptors β1, β2, and α1 were evaluated in the context of adrenergic blockade. The expression of key regulators of extracellular matrix (ECM) turnover (matrix metallopeutase 2 (MMP2) and tissue inhibitor of metallopeutase 1 (TIMP1) were also evaluated. The expression of lysyl oxidase was studied considering its major role in collagen fiber cross-linking. Finally, the expression of transforming growth factor β1 and β2 (TGF-β1 and TGF-β2) and connective tissue growth factor (CTGF) were also studied because they are closely related to collagen and fibronectin production by myocardial fibroblasts.

Staining for Capillary Density Measurement

Sections of 8-μm thickness were cut from the frozen LV and were stained with isoelectric B4 from Bandeiraea simplicifolia (Aublet) with horseradish peroxidase (Sigma, Mississauga, Ontario, Canada), and capillary density was analyzed in the subendocardial region of the LV myocardium (inner third). Pictures of 3 different LV fields of 8 animals per group were taken at ×200 magnification. The number of capillaries per field was measured for each 3 fields and reported as a mean for each animal. The observer was blinded for the groups during the analysis.

Statistical Analysis

Results are presented as mean±SEM. Intergroup comparisons were done using 2-way ANOVA followed by Bonferroni post-test if interaction between disease (AR) and treatment (carvedilol) was significant. The Student t test was used when 2 groups were compared directly. Statistical significance was set at probability values <0.05. Data and statistical analysis were performed using Graph Pad Prism version 5.02 for Windows, Graph Pad Software (San Diego, CA).

Results

Clinical Data

Carvedilol treatment was well tolerated. Four animals died in the nontreated AR group and 3 in the AR-carvedilol group.

Echocardiography

A complete M-Mode, 2D, and Doppler echocardiogram was performed on the animals under 1.5% isoflurane anesthesia using a 12-MHz probe with a Sonos 5500 echograph (Philips Medical Imaging, Andover, MA) immediately before and during surgery and after 6 months. An echocardiogram after 2 weeks was also performed to quantify AR before starting drug treatment to make sure all animals still met the entry criteria. Left ventricular dimensions, wall thickness, ejection fraction, diastolic function, cardiac output (ejection volume in the left ventricular outflow tract×heart rate) were evaluated as previously reported. AR was semiquantified at each time point as described in the previous section. Animals had to meet all the criteria of severe AR by semiquantification at each time point to remain included in the protocol.

Hemodynamic Measurements

LV end-diastolic pressures and dP/dt (positive and negative) were measured invasively using a dedicated 2F impedance catheter (Millar Instruments, Houston, TX) under 1.5% isoflurane anesthesia after 6 months.

divided into 2 groups (n=12 per group): (1) AR untreated and (2) AR treated with carvedilol 30 mg/kg/d orally. Twenty sham-operated rats were used as controls and randomly assigned to the following groups: 3 sham-untreated and 4 sham-treated with 30 mg/kg/d of carvedilol. AR was considered severe by echocardiography by the presence of all of the following criteria at the time of surgery: color Doppler ratio of regurgitant jet width to LV outflow tract diameter >50%, retrograde holo-diastolic flow in proximal descending aorta with end-diastolic velocity >18 cm/s, ratio of time-velocity integral of reversed diastolic flow to forward systolic flow in descending thoracic aorta >60%, and acute increase in LV diastolic dimension during the surgical procedure. Echocardiographic criteria of AR severity had to be accompanied by an acute drop of aortic diastolic pressure of at least 30% to qualify. Animals not meeting the echographic and hemodynamic criteria were not included in the study. Drug treatment was started 2 weeks after the surgical procedure to allow recovery from the acute phase and continued for 6 months. Animals were clinically evaluated daily by experienced animal laboratory technicians for the presence of signs of heart failure (increased respiratory rate/distress and/or peripheral edema) and were weighed weekly. At the end of the protocol, surviving animals were euthanized, hearts were quickly dissected, and all cardiac chambers were weighed. LVs were snap-frozen in liquid nitrogen and kept at −80°C for further analysis. This protocol was approved by the Laval University Animal Protection Committee according to the recommendations of the Canadian Council on Laboratory Animal Care.
Those deaths were sudden, occurred during the night in the awake/active cycle of the rats, and were not preceded by signs of heart failure. There was no clinical heart failure in any of the animals. Body weight and tibial length (an index of growth) were similar in all groups (Table 1).

Cardiac Hypertrophy
Hearts were explanted at the end of the protocol and cardiac chambers were weighed. Results are summarized in Table 1. Carvedilol had no impact on the heart weight of the sham control animals. Animals with AR (treated or not) had severe cardiac hypertrophy, as shown by their increased total heart weight but carvedilol treatment significantly reduced LV, right ventricular, and left atrial hypertrophy.

Echocardiographic Data
The echocardiographic data obtained in anesthetized animals after 6 months of treatment are summarized in Table 2. AR severity was similar in both AR groups. As expected, AR resulted in significant end-diastolic and end-systolic dilatation and eccentric remodeling (decreased relative wall thickness, RWT). Ejection fraction remained in the normal range in AR animals, although it was slightly lower than in sham groups. Carvedilol treatment in AR reduced end-diastolic and end-systolic diameters compared with untreated AR. Ejection fraction was also improved as well as the myocardial performance index.

Hemodynamic Data
Hemodynamic data obtained after 6 months in anesthetized animals are summarized in Table 3. Heart rate was lower in both carvedilol groups (sham and AR). Stroke volume was not significantly affected by carvedilol in the AR group. The lower heart rate combined with an unchanged stroke volume resulted in a reduced calculated cardiac output in the carvedilol-AR compared with untreated AR. Systolic blood pressure was similar in all 4 groups. As expected in this disease, both AR groups had a lower diastolic pressure and therefore an increase in pulse pressure. This was not affected by carvedilol treatment. There were no significant effects of carvedilol on dP/dt positive or negative values. LV end-diastolic pressure was increased in the AR group and was normalized by carvedilol treatment.
The relative expression of ANP, BNP and Follistatin-like protein 1 (Fstl1) mRNAs were measured after 6 months in specifically preserved LV tissues. Results are reported in Figure 1. All AR groups displayed a significant increase in ANP mRNA expression, as shown in the top panel of Figure 1. This overexpression was significantly decreased by carvedilol treatment. BNP and Fstl1 expressions were also significantly increased in untreated AR rats, and their expression was unaffected by carvedilol treatment.

**ECM Remodeling Gene Expression**

Results for the mRNA relative expression of collagen I, collagen III, and fibronectin in LV tissue are shown in Figure 2. Collagen I mRNA expression (top) was increased in untreated AR animals. Carvedilol significantly reduced this overexpression of collagen I mRNA. Carvedilol significantly reduced the overexpression of collagen I mRNA. Collagen III and fibronectin mRNA expressions were significantly increased in AR, but carvedilol did not have any effect on those expressions.

The level of mRNA expression of other components of the ECM regulation (lysyl oxidase 1 [LOX1], MMP2, TIMP1, TGF-β1, TGF-β2, and CTGF) was evaluated. Results are summarized in Figure 3. All expressions were increased in the AR groups compared with sham controls. Carvedilol tended to reduce the overexpression of LOX1 and TIMP1, whereas there were no significant effects on the other factors.

**Adrenergic Receptors**

The results of mRNA expression of adrenergic receptors β1, β2, and α1 in LV tissues are shown in Figure 4. AR significantly reduced the mRNA expression of β1-receptors in the LV tissue, and normal levels were restored by carvedilol treatment. Although AR had no significant impact on β2-receptor expression, carvedilol treatment significantly increased its expression. Myocardial α-receptor mRNA expression was lower in both AR groups without any significant measurable effect of carvedilol treatment.
Capillary Density in the LV Myocardium
The density of capillaries was measured in LV tissue and results are summarized in Figure 5. The LVs of AR animals had a significantly lower capillary density compared with both sham groups. Carvedilol treatment tended (P=0.08) to normalize capillaries density in AR animals.

Discussion
The results of this study clearly demonstrate many beneficial effects of carvedilol treatment in an experimental model of severe aortic valve regurgitation with dilated hearts but normal LV ejection fraction. AR rats treated with carvedilol for 6 months had less LV hypertrophy, less LV dilatation, improved ejection fraction, and lower LV filling pressures. The mild bradycardia induced by carvedilol did not result in an increase in AR severity or stroke volume. On the contrary, this mild bradycardia resulted in a decrease of cardiac output and consequently of the volume overload burden endured by the LV per minute. Carvedilol-treated AR rats also had lower LV ANP and collagen I mRNA levels. β-Adrenergic receptor expression was also improved by carvedilol as well as LV capillary density. Carvedilol was well tolerated, and its beneficial effects were clear despite that the drug induced a slightly lower heart rate.

The issue of finding the best treatment for chronic aortic valve regurgitation remains unresolved. Vasodilators have been investigated in many small clinical trials, but results have been inconsistent. The most recent treatment guidelines do not support the use of vasodilators to treat chronic AR with normal ejection fraction.1 An overactivation of the adrenergic system has been reported in chronic AR models,10–15 and this finding was the basis of our previous studies of metoprolol in an experimental model of the disease. In line with our findings, Sampat et al7 published in 2009 the results of a study of a retrospective cohort of 756 patients suggesting that β-blockers are beneficial for survival in patients with chronic AR. The attached editorial suggested that the recommendations not to use β-blockers in patients with AR should be removed from the treatment guidelines and that prospective large scale studies should be designed to test the effects of β-blockers in AR appropriately.16 Nevertheless, fear still remains in the clinical world that β-blocker–induced bradycardia might prolong diastole and increase regurgitation time, even though this statement is not supported by any solid experimental data. It is known that patients with LV dysfunction from chronic AR respond well to β-blocker therapy after aortic valve replacement.17 The safety of β-blockers in patients with AR from Takayasu arteritis has been reported in a few small Japanese studies in the past, but clinical data about β-blockers in AR remain very limited.17–19

The data we obtained in the present carvedilol study are consistent with our previous ones with metoprolol.5,6 This suggests a positive class effect of β-blockers to protect against volume overload cardiomyopathy induced by chronic AR while LV ejection fraction is still within normal range (in prevention of heart failure). It is noteworthy that the beneficial effects of carvedilol were present despite a relative bradycardia induced by the drug (mean, –20 bpm). This decrease in heart rate was not accompanied by an increase in AR severity or stroke volume. Although the bradycardia was
could have been missed. Peripheral resistance were not performed, and small effects of carvedilol on these parameters would have been detected in the awake or exercising animals is a possibility that has not been assessed in this protocol.

The protective effects of carvedilol were not complete: LV hypertrophy was not totally prevented, LV dimensions did not return to normal range, BNP and Fstl1 levels remained unaffected by carvedilol, suggesting that the stress on the LV has not been completely overcome. This was expected because significant volume overload remains present and cannot be eliminated unless mechanically corrected by valve surgery. Nevertheless, the LV remodeling process was clearly slowed, and many hemodynamic parameters were significantly improved as well as the expression of many tissue components of related to myocardial remodeling. Collagen I and LOX1 as well as TIMP1 overexpressions were reduced by carvedilol treatment. This suggests that carvedilol had protective effects against ECM remodeling. Carvedilol also strongly tended to improve myocardial capillary density. This increase in myocardial capillary density may have consequently improved oxygen and metabolic fuel availability and delivery to the cardiomyocytes. This may have led to optimized myocardial energetic metabolism, improved diastolic and systolic performance, and decreased susceptibility of the LV to fibrosis, arrhythmia, and subendocardial ischemia. In another animal model of the disease, bradycardia pacing improved capillary density and myocardial performance. However, the exact mechanisms involved are not well understood.

In conclusion, carvedilol clearly exerts protective effects against volume-overload cardiomyopathy in this animal model of chronic aortic valve regurgitation with dilated hearts but normal ejection fraction. Although the protective effects of carvedilol were not complete, we did not find overall any parameters in vivo or on tissue analysis that were negatively affected by carvedilol treatment. These results are in line with previous data suggesting a positive class effect of β-blockers. Combined with the recent publication by Sambat et al strongly suggesting positive effects in humans, the fear of using β-blockers in the context of AR may be unjustified. Prospective, carefully designed clinical trials testing the effects of β-blockade in AR must be done to assess this potential new treatment avenue in a disease currently lacking any proven effective medical treatment.

Sources of Funding
This work was supported by operating grants to Drs Couet and Arsenaull from the Canadian Institutes of Health Research (MOP-61818 and MOP-106479) and the Quebec Heart Institute Corporation.

Disclosures
None.
The medical treatment of asymptomatic patients with severe aortic valve regurgitation (AR) remains controversial. No pharmacological treatment has been clearly shown to be effective to protect the myocardium against the deleterious effects of chronic volume overload. Despite the recent publication of promising human data, β-blockade in chronic AR remains controversial because of the deleterious effects of bradycardia. More data are needed to support this potentially new treatment strategy. We hypothesized that carvedilol might be a safe treatment option in chronic AR, considering its β-blocking and α-blocking effects and proven efficacy in patients with established heart failure. We have designed a study in a rat model of chronic AR testing the efficacy of carvedilol at maintaining cardiac function and slowing the development of eccentric left ventricular hypertrophy over 6 months, starting treatment 2 weeks after surgical AR induction. Carvedilol treatment resulted in less left ventricular dilatation. Ejection fraction was improved and filling pressures were reduced by carvedilol. β1-Adrenoreceptor expression was also improved. Those beneficial effects were noted despite the presence of drug-induced bradycardia. The results of this study revealed that carvedilol exerted protective effects against volume-overload cardiomyopathy in this model of AR with preserved ejection fraction. These results, in addition to those shown previously with metoprolol, suggest a protective class effect of β-blockers. Combined with the recent publication of promising human data, our findings support the need to carefully design a prospective study in humans to evaluate the effects of β-blockers in chronic AR.

CLINICAL PERSPECTIVE

References


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*Circ Heart Fail.* 2011;4:207-213; originally published online January 7, 2011;
doi: 10.1161/CIRCHEARTFAILURE.110.958512
*Circulation: Heart Failure* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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Table S1. QuantiTect® Primer Assays used in Q-PCR analysis of gene expression.

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