Title: Benefits of long-term beta-blockade in experimental chronic aortic regurgitation

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Running head: Beta blockade in chronic aortic regurgitation

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Abstract

Objectives: To assess the long term effects of beta blockade on survival and LV remodeling in rats with aortic valve regurgitation (AR).

Background: The pharmacological management of chronic AR remains controversial. Until now, no drug has been definitively proven effective to delay the need for valve replacement or to affect morbidity and/or mortality. Our group has reported that the adrenergic system is activated in an animal model of AR and that adrenergic blockade may help maintain a normal left ventricular (LV) function. The effects of a prolonged treatment with a beta-blocker are unknown.

Methods: 40 Wistar rats with severe AR were divided in 2 groups (n=20/gr) treated or not with metoprolol (MET) 25 mg/kg/day for 1 year. LV remodeling was evaluated by echocardiography. Survival was assessed by Kaplan-Meir curves. Hearts were harvested for tissue analysis.

Results: All animals treated with MET were alive after 6 months compared with 70% of untreated animals. After 1 year, 60% of MET animals were alive vs. 35% untreated (p=0.028). All but 1 death were sudden. There were no differences in LVEF (all above 50%) or LV dimensions. LV mass tended to be smaller in the MET group. There were significantly less sub-endocardial fibrosis in this group as well as lower LV filling pressures (LVEDP). Beta adrenergic receptor ratio (β1/β2) was improved in MET treated animals.

Conclusion: A 1-year treatment with metoprolol was well tolerated in rats with severe AR. Metoprolol treatment improved 1 year survival, minimized left ventricular hypertrophy, improved LV filling pressures, decreased LV sub-endocardial fibrosis and helped restore beta adrenergic receptor ratio.
**Key words:**
aortic valve regurgitation
volume overload
beta blockers
adrenergic system
Introduction:
Severe aortic regurgitation (AR) is associated with a long asymptomatic period during which the left ventricle (LV) progressively dilates and hypertrophies in response to a chronic volume overload. Paralleling LV dilatation, LV function will eventually decrease, symptoms will appear and valve replacement surgery will become inevitable. In the past decades, several investigators have reported data suggesting that medical therapy with vasodilators such as nifedipine, hydralazine or angiotensin converting enzyme inhibitors (ACEI) may be effective to reduce the aortic regurgitant volume and help maintain left ventricular function (3). Nifedipine, a calcium channel blocker, seemed especially promising (20). However, the results of a recently published trial by Evangelista et al. have failed to confirm these positive effects of nifedipine or ACEI treatment compared to a placebo (7). This ongoing controversy regarding the medical management of chronic severe AR has transpired in the latest AHA/ACC Valvular Heart Disease Treatment Guidelines in which vasodilators are no longer recommended for the medical management of chronic AR in patients with normal ventricular function (4). No drug has yet been clearly shown to be effective to slow LV dilatation, hypertrophy, loss of systolic function or have any impact on morbidity and mortality in chronic AR.

Focusing on a different target, our group has previously reported in an animal model of chronic AR that the adrenergic system is abnormally activated and that blocking this system seems to play an important role in preventing LV dilatation, hypertrophy and loss of systolic function (19). The current study was primarily designed to evaluate if those beneficial effects of beta-blockade on LV function and remodeling would persist and translate into a survival benefit in rats with severe aortic valve regurgitation in the long term.
Material and Methods

Animal model of aortic regurgitation

40 male Wistar rats (300-350g, Charles River, Qc, Canada) had severe AR induced by retrograde puncture of the aortic valve leaflets as previously described (2, 17-19) and randomly divided in 2 groups (n=20/gr) as follows: 1) untreated AR (AR) and 2) AR receiving 25 mg/kg/day of metoprolol (MET) (Sigma, Oakville Ont. Canada) in drinking water. The dosage of metoprolol was similar to the one used previously (19). Significant physiologic beta-blockade was previously confirmed by the blunted response of heart rate to adrenergic stimulation by dobutamine infusion. 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 LVOT 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 with metoprolol was started 2 weeks after the surgical procedure to allow for recovery and continued for 12 months thereafter. 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 sacrificed, hearts were quickly dissected and all cardiac chambers were weighed. LV were snap-frozen in liquid nitrogen and kept at -80° Celsius for further analysis. This protocol was approved by the
Université Laval’s Animal Protection Committee according to the recommendations of the Canadian Council on Laboratory Animal Care.

**Echocardiography**

A complete M-Mode, 2D and Doppler echocardiogram was performed on the animals under 1.5% inhaled isoflurane anesthesia using a 12 MHz probe with a Sonos 5500 echograph (Philips Medical Imaging, Andover, MA) immediately before and during surgery, at 2 weeks and after 6 and 12 months. The echocardiogram after 2 weeks was 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 X heart rate) were evaluated as previously reported. AR was semi-quantified at each time-point as described in the previous section. Animals had to meet all the criteria of severe AR by semi-quantization at each time-point to remain included in the protocol.

Hemodynamic measurements: Left ventricular pressures and dP/dt (positive and negative) were measured invasively using a dedicated catheter under 1.5% isoflurane anesthesia after 12 months in survivors. At other times during the protocol, systolic and diastolic blood pressures were measured non-invasively using the tail-cuff method. Metoprolol was continuously available in drinking water and was not stopped before the measurements were performed. All experiments were performed at the same period of the day (morning).

**Tissue analysis**

*Cardiomyocyte cross-sectional area (CSA) and evaluation of LV fibrosis*
Sections from paraffin-embedded mid-LV portions were stained using Trichrome-Masson coloration. Three sub-endocardial sections/slide from all surviving animals were analyzed for the evaluation of cross sectional area (CSA) of the cardiomyocytes as previously described (2, 17-19). These sections were also used to evaluate the proportion of LV sub-endocardial fibrosis as the blue (fibrosis)/red (myocytes) ratio using a computerized image analysis system (Image-Pro Plus, Version 4.5, Media Cybernetics, Silver Springs, MD). The sub-endocardial sections were defined as the inner third of the LV wall facing the LV cavity.

*Semi-quantitative analysis of mRNA accumulation by RT-PCR*

Total RNA extraction from the LV, reverse transcription and DNA amplification by PCR of collagens type I and III, fibronectin and pro-metalloprotease 2 (proMMP2) were performed as described previously (17-19).

*Analysis of mRNA accumulation by quantitative RT-PCR*

Tissues stored frozen in RNAlater® (Ambion, Austin, TX) were homogenized in Trizol (Invitrogen) using a Polytron according to the standard Trizol procedure. Fifty ng of RNA was converted to cDNA using the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA), a procedure which includes a genomic DNA elimination step. The cDNA obtained was further diluted 10-fold with water prior to amplification (with the final concentration corresponding to 0.25 ng/µl of initial RNA). 1.25ng of diluted cDNA was amplified in duplicate (*technical* duplicates) by Q-PCR in a Rotor-Gene™ 6200 thermal cycler (Corbett Life Science, Sidney, Australia), using the QuantiTect® SYBR Green PCR kit and QuantiTect® Primer Assays (pre-optimized specific primer pairs from Qiagen; Table 1). Each run included one tube with water only (no template control), one
tube with a representative RNA sample (no RT control), and a series of 10-fold dilutions of a representative cDNA sample to confirm the efficiency of the amplification reaction. The quantification of gene expression was based on the \(-2^{\Delta\Delta C_t}\) method (12). Briefly, mean Ct values of technical duplicates for each gene of interest were subtracted from the mean Ct value (hence \(\Delta C_t\)) of the control “housekeeping” gene beta-2-microglobulin. The differences in the mean \(\Delta C_t\)s between groups of rats (\(\Delta\Delta C_t\)) allow the calculation of relative levels of induction/repression of genes of interest.

**Statistical analysis**

Survival was analyzed by standard Kaplan-Meier analysis with log-rank test. Other results are presented as mean ± SEM unless specified otherwise. Inter-group comparisons were done using unpaired t tests and intra-group comparisons with paired t tests. Statistical significance was set at a \(p<0.05\). Data and statistical analysis were performed using Graph Pad Prism version 4.02 for Windows, Graph Pad Software (San Diego CA).

**Results**

Survival:

Metoprolol treatment was well tolerated. Figure 1 shows the survival curves of animals treated or not with metoprolol (MET). All MET animals were alive after 6 months compared with only 70% in the untreated group. After one year, the survival of MET animals was significantly better with a survival rate of 60% compared to 35% for the untreated group (\(p=0.045\)). With the exception of one animal which developed clinical
signs of heart failure in the metoprolol group, all deaths were sudden and occurred during the night (rats’ active period).

Hemodynamic data:
Hemodynamic data are summarized in table 2. There were no significant differences in body weight, resting heart rate, cardiac output, systolic pressure or pulse pressure after 6 or 12 months between both groups. Beta-blockade was confirmed previously by the lower maximal heart rate response to an intravenous dobutamine infusion of 20 μg/kg/min in metoprolol treated rats (Met) compared to untreated animals (AR) despite similar resting heart rates (max HR AR: 452 ± 12 bpm vs Met: 415 ±14 bpm, p=0.05). Invasive measurements showed no difference in positive and negative dP/dt between groups at the end of the protocol. However, left ventricular end-diastolic pressures (LVEDP) were significantly lower in the metoprolol group vs untreated AR as shown in table 2. LVEDP values in the metoprolol group were similar to those of normal-sham animals (Met: 8±1.2 mmHg vs Sham: 9±1.0 mmHg, p=0.71).

Echocardiographic data:
Echographic data are summarized in table 3. AR severity was reassessed at each echocardiographic examination and remained similar between both groups (data not shown). There were no significant differences in left ventricular diastolic or systolic diameters between groups at 6 and 12 months. Left ventricular ejection fraction remained above 50% in all groups. After 12 months, LV ejection fraction tended to be slightly better in the MET group vs. no treatment (57.3% ± 2.23% vs. 52.7% ± 1.80%, p=0.16). 4/12 rats in the MET group had and ejection fraction still above 60% after one year versus none (0/7) in the untreated group.
A sub-group analysis of echocardiographic data after 6 months showed that the animals that would survive the whole one year protocol had similar end-diastolic diameters (EDD) but smaller end-systolic (ESD) diameters than the animal that would die before the end of the protocol (survivors EDD vs. non-survivors: 11.5±0.24 mm vs. 12.3±0.21 mm, p=0.22; survivors ESD vs. non-survivors: 7.1±0.31 mm vs. 8.3±0.30 mm, p=0.012).

Measurement of left ventricular hypertrophy:
Measured LV weights (by echocardiography) were similar between groups after 6 months. We found a strong trend towards smaller LV weights (weighed explanted hearts) in the MET compared to untreated group after 12 months (MET: 1695 g ± 92.3 vs. untreated: 1915 g ± 59.9; p=0.07). Cardiomyocyte cross-sectional area (CSA) also tended to be smaller CSA in the MET group (MET: 50 A.U. ± 2.2 vs. untreated: 55 A.U. ± 3.1) but this difference did not reach statistical significance (p=0.14). There were no differences in lung or liver weight between groups (results not shown).

LV fibrosis and extracellular matrix remodeling:
Results for the semi-quantification of sub-endocardial LV fibrosis are shown in figure 2 (top). Although there were no significant differences between groups after 6 months (19), a significant decrease in the amount of sub-endocardial fibrosis was clearly evident in the MET group after 1 year (p=0.009). The LV expression of mRNA for fibronectin, collagen1, 3 and matrix metalloprotease 2 (MMP2) revealed no differences between groups after 1 year (results not shown). Figure 2 (bottom panels) shows typical examples of Trichrome-Masson staining of the LV demonstrating increased sub-endocardial fibrosis in the untreated group compared to those treated with metoprolol.
Myosin heavy chains (MHC) mRNA analyses:
Real-time quantitative RT-PCRs evaluating the modulation of both $\alpha$ and $\beta$ myosin heavy chains (MHC) are illustrated in Figure 3. AR animals had significantly lower levels of mRNA encoding the $\alpha$MHC and higher levels of $\beta$MHC compared to normal rats. MET treatment had no significant effect on this parameter.

$\beta$-adrenergic receptors:
The gene expression of both $\beta_1$ and $\beta_2$ adrenergic receptors in the LV of surviving animals by qRT-PCR were evaluated (Figure 4). mRNA levels of $\beta_1$ adrenergic receptors were down-regulated in untreated AR rats while MET treatment tended to normalize the situation. Opposite results were found for $\beta_2$ receptors. MET treatment therefore improved the $\beta_1/\beta_2$ ratio mRNA levels.

Two other components of the $\beta_1$ adrenergic receptor pathway namely the Gs protein $\alpha$-subunit and the catalytic $\alpha$-subunit of the protein kinase A (PKA) were analyzed. As depicted in Figure 5, mRNA levels encoding the Gs$\alpha$ protein were higher in AR animals but remained unchanged in MET-treated rats. mRNA levels for PKA decreased in AR animals compared to sham group while those of MET rats were not significantly different from controls.

Discussion
The management of chronic volume overload such as chronic aortic valve regurgitation remains controversial and widely debated. Vasodilators, mainly nifedipine, have been
the cornerstone of pharmacological therapy of those patients for the past decades (3). However, the use of nifedipine and other vasodilators in AR was based on data which unfortunately could not be reproduced in a recently published placebo-controlled trial comparing nifedipine or enalapril in AR patients (7, 20). Consequently, those drugs have lost a lot of credibility and many physicians are now skeptical that any pharmacological treatment can be effective in chronic AR.

The rationale for vasodilator use in AR was to help decrease regurgitant volume, preload and afterload. Our group has been evaluating a completely different target in chronic AR by studying the role of the adrenergic system in an animal model in rats (19). In the present study, we report that a one-year treatment with a beta-adrenergic blocker (metoprolol) improves survival in rats with severe aortic valve regurgitation. Despite its relatively low dosage for rats (25 mg/kg/min) metoprolol resulted in a significant level of physiologic beta-blockade as shown by the blunted heart rate response to adrenergic stimulation by dobutamine and significant effects on beta-adrenergic receptor ratio. To our knowledge, it is the first time that such an observation is reported. The role of beta-blockade in the management of aortic regurgitation has been explored by other investigators in animal models (1, 19, 21, 23-27) (table 4). Those studies suggested that adrenergic blockade may be beneficial in animal models of acute aortic regurgitation although none of them had evaluated the long term effects in a chronic AR model. Table 5 summarizes the studies performed in humans related to the hypothesis of the role of the adrenergic system or beta-blockade in AR. Again, there have been no long term large prospective studies specifically testing the effects of adrenergic blockade in chronic AR in humans. Significant alterations of beta-adrenergic receptors have been shown on myocardial biopsies of AR patients (6, 9). One study showed that beta-blockers can be safely given after valve replacement surgery in humans with AR to help
left ventricular function recovery (14). Only two small observational studies on Japanese women with severe AR and Takayasu arteritis showed that beta-blockers were well tolerated and that they may even reduce the progression of left ventricular hypertrophy (10, 16). Clearly, no hard conclusions can be derived from these data but the conclusions of these small trials as well as previous animal studies all seem to support our hypothesis that the adrenergic system is involved in the myocardial remodeling associated with AR. None of these studies were designed to evaluate the effects of beta-blockers on survival.

In our study, death occurred suddenly without any warning signs of impending heart failure. There was a clear decrease in sub-endocardial fibrosis and a strong trend towards less LV hypertrophy in animals treated with metoprolol. Myocardial fibrosis and hypertrophy are factors known to increase the risk of sudden death in other cardiac diseases. All animals treated or not with metoprolol had an ejection fraction very close to or above 50% at the end of the protocol and were not in heart failure even though their left ventricles were significantly dilated. These results raise the hypothesis that myocardial fibrosis may be pro-arrhythmogenic in AR and that the deaths of the animals in our protocol were arrhythmic in nature. Clearly, they did not die of progressive heart failure. A sub-group analysis showed that survivors had smaller left ventricles at mid-term of the protocol. However, ejection fractions were similar and close to normal after 6 months and 12 months in survivors thereby lending further support to the anti-arrhythmic hypothesis rather than a anti-heart failure effect.

Beta-blockers have been proven effective to decrease the risk of sudden death in other cardiac diseases such as congestive heart failure by counter-acting the negative effects of an over-activated adrenergic system and restoring the sympathetic-parasympathetic balance. Beta-blockers may have acted in the same way in our animals with severe
chronic AR and preserved LV ejection fraction. Indeed, we observed that metoprolol improved the ratio of gene expression of the $\beta_1$ and $\beta_2$ adrenergic receptors which was shifted towards less $\beta_1$ and more $\beta_2$ in untreated AR animals.

Our study was unfortunately underpowered to clearly detect a statistically significant difference in left ventricular ejection fraction or hypertrophy between groups. A survivor bias was probably present. However, ejection fraction, LV mass and myocyte cross-sectional area all tended to be positively affected by metoprolol treatment (better LVEF, smaller LV mass and myocyte CSA). Filling pressures (LVEDP) were improved by metoprolol.

Myocardial contractility is affected by multiple factors interacting with each other. Myosin heavy chains are one key component of myocardial contractility (8). Rodent hearts mostly express $\alpha$ MHC with a high $\alpha/\beta$ MHC ratio and most cardiomyopathy models induce a shift in this ratio (decreased $\alpha$ and increased $\beta$). This shift was never documented in a model of chronic aortic valve regurgitation and we therefore do so for the first time. Moreover, the adrenergic system is a key determinant in the expression of myosin subtypes, therefore in direct link with our main hypothesis. Adrenergic stimulation favors $\alpha$ MHC expression whereas $\beta$ blockade will induce $\beta$ MHC. Based on previous studies by other investigators in other animal models (mostly transgenic mice), it was though that hearts expressing more $\beta$ MHC would cope less well and develop more hypertrophy in response to chronic stress (8). In this study we show that $\beta$ blockade does not restore a normal $\alpha/\beta$ MHC ratio and even tends to increase the level of $\beta$ MHC. Despite the tendency to increased $\beta$ MHC, the animals survived longer and tended to have less hypertrophy. These findings do not support the hypothesis that increased $\beta$ MHC is deleterious in our model. It is known that $\beta$ MHC has the ability to
generate contractile force with less energy consumption than α MHC. We suggest that this may be a protective mechanism against chronic stress in AR. This hypothesis should be investigated in upcoming studies focusing on myocardial metabolism in chronic AR.

It is interesting to note that despite their relatively preserved ejection fraction measured by echocardiography, the left ventricles of animals with AR displayed significant amounts of sub-endocardial fibrosis and that invasive measurement of dP/dt unmasked some contractile dysfunction not detectable by echocardiography in both groups. Again, our study was underpowered to detect any difference between treated and untreated groups. However, we did find a significant improvement of LVEDP in metoprolol treated animals compared to untreated AR animals. Filling pressures in the metoprolol group were similar to normal sham operated animals. This finding combined with less sub-endocardial fibrosis demonstrates a beneficial effect of beta-blockade on diastolic properties of the LV. Fibroblast proliferation and collagen secretion is regulated by the adrenergic system (5). Fibroblasts express β-adrenergic receptors on their cell membranes and beta blockers have been shown to reduce myocardial fibrosis. Therefore, our results that beta blockers would reduce myocardial fibrosis were expected. However, expressions of collagens as well as fibronectin and MMP2 were abnormally high after 6 months (19) whereas we did not find any significant alterations after 12 months. It has been shown by others that a decreased degradation is the most important factor for the accumulation of fibrosis in AR. Our results suggest that between 6 and 12 months, the fibrotic process has ended or reached equilibrium as stated earlier.
There are no large-scale studies who evaluated the natural history of patients with AR with normal ejection fraction in a homogeneous comparable cohort. Current data is clearly imperfect and is derived from the summary of many small series very heterogeneous in nature who studied 30 to about 100 patients each with a follow up ranging from 3 to 14 years (4). In those studies, mortality rates were low. However, the latest 2006 ACC/AHA guidelines point out that one quarter of patients who die or develop systolic dysfunction do so before the onset of any warning symptoms. Sudden death, although relatively rare, has been reported in asymptomatic patients with normal left ventricular ejection fraction. Ventricular arrhythmias have been reported in patients with aortic valve regurgitation and were correlated with the degree of ventricular enlargement (11, 13, 15, 22). Those studies were not designed however to address the issue of mortality in patients displaying more ventricular arrhythmias. Our study suggests that animals with severe AR experienced sudden arrhythmic deaths and that treatment with a beta-blocker is effective to decrease mortality. Further studies will be performed to better address this hypothesis.

Clearly, the results of a study performed on animals cannot be directly transposed to humans. The dose of metoprolol given to the animals was relatively small for a rat and did not induce any significant resting bradycardia. Whether the same results would have been obtained in the presence of bradycardia using larger doses of metoprolol is unknown. Heart rate and all other hemodynamic parameters were evaluated in anesthetized rats: the effects on hemodynamics while the animals were awake are unknown. Many unanswered questions remain before this treatment can be tested in humans.
In summary, we report for the first time that a long-term treatment with metoprolol decreases sudden death in a rat model of chronic aortic valve regurgitation with relatively preserved LV ejection fraction, decreases filling pressures and sub-endocardial myocardial fibrosis and helps improve myocardial beta-adrenergic receptors ratio. Further studies are needed to better understand these beneficial effects of blocking the adrenergic system in chronic aortic valve regurgitation.
Acknowledgments: none

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Disclosures: none
References


**Figure legends**

**Figure 1**: Survival Kaplan-Meier curves of rats with severe chronic aortic valve regurgitation treated (MET; white circles) or not (AR; black circles) with metoprolol.

**Figure 2**: Left ventricular fibrosis and extra-cellular matrix (ECM) remodeling after 12 months. Top: Quantification by blue/red ratio from trichrome-Masson stained LV sections. Bottom: Typical examples of trichrome-Masson stained sub-endocardial LV sections. Collagen fibers (blue); cardiomyocytes (red), magnification: X200. top: untreated AR; bottom: treated with metoprolol (MET).

**Figure 3**: Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of myosin heavy chains (MHCs) after 12 months. From top to bottom: αMHC, βMHC and α/β ratio of mRNA levels. Results are reported in arbitrary units (AU) as mean ± SEM (n=7-9/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR: untreated and MET: group treated with metoprolol. *: p<0.05 vs sham  **: p<0.01 vs sham

**Figure 4**: Real-time quantitative RT-PCR of the LV mRNA levels of the β adrenergic receptors after 12 months. From top to bottom: β1, β2 and ratio of β1/β2 mRNA levels. Results are expressed in arbitrary units (AU) as mean ± SEM (n=7-9/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR: untreated and MET: group treated with metoprolol. *: p<0.05 vs sham  **: p<0.01 vs sham  #: p<0.05 vs AR
Figure 5: Real-time quantitative RT-PCR of the LV mRNA levels of the Gs α-subunit and the protein kinase A catalytic α-subunit. Results are expressed in arbitrary units (AU) as mean ± SEM (n=7-9/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR: untreated and MET: group treated with metoprolol. *: p<0.05 vs sham **: p<0.01 vs sham
Table 1: QuantiTect® Primer Assays used in Q-PCR analysis of gene expression.

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Table 2. Hemodynamic data after 6 and 12 months.

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Measurements obtained under inhaled 1.5% isoflurane anesthesia. AR: untreated group; MET: group treated with metoprolol. HR: heart rate; SV: stroke volume in left ventricular outflow tract by pulsed Doppler; CO: cardiac output (SV X HR); SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure (SBP-DBP); dP/dt_{min}: minimal derivative of pressure/time; dP/dt_{max}: maximal derivative of pressure/time; LVEDP: left ventricular end-diastolic pressure; na: non applicable. Values are mean ± SEM of the indicated number of surviving animals per group with the exception of for the dP/dt and LVEDP values (n=5).
Table 3. Echocardiographic data after 6 and 12 months.

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</tr>
<tr>
<td>EDPWT, mm</td>
<td>2.2 ± 0.04</td>
<td>2.1 ± 0.02</td>
<td>0.72</td>
<td>2.2 ± 0.04</td>
</tr>
<tr>
<td>EF, %</td>
<td>57 ± 1.9</td>
<td>60 ± 2.3</td>
<td>0.31</td>
<td>53 ± 1.8</td>
</tr>
</tbody>
</table>

AR: untreated group; MET: group treated with metoprolol. EDD: end-diastolic diameter; ESD: end-systolic diameter; SWT: septal wall thickness; PWT: posterior wall thickness, EF: left ventricular ejection fraction. Values are mean ± SEM of the indicated number of surviving animals per group.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year published</th>
<th>Ref. in text</th>
<th>Animal model</th>
<th>Intervention</th>
<th>Results summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anzai et al</td>
<td>1996</td>
<td>(1)</td>
<td>Rabbit AR Acute</td>
<td>Pharmacologic sympathetic denervation</td>
<td>Preservation of normal βAR signalling by pharmacologic denervation</td>
</tr>
<tr>
<td>Plante et al</td>
<td>2004</td>
<td>(19)</td>
<td>Rat AR chronic</td>
<td>Metoprolol 25/mg/kg/d for 6 months</td>
<td>Metoprolol improved LVEF Decreased LVH Increased β AR expression</td>
</tr>
<tr>
<td>Suzuki et al</td>
<td>1997</td>
<td>(21)</td>
<td>Rabbit AR Acute</td>
<td>IV propanolol 7 days</td>
<td>Propanolol prevented βAR down-regulation</td>
</tr>
<tr>
<td>Wright et al</td>
<td>1989</td>
<td>(23)</td>
<td>Rabbit AR 8 weeks</td>
<td>Bradycardial pacing</td>
<td>Bradycardial pacing improved myocardial capillary density and cardiac work/minute in AR</td>
</tr>
<tr>
<td>Xiong et al</td>
<td>1995</td>
<td>(24)</td>
<td>Rabbit AR Acute (14 days)</td>
<td>Propanolol</td>
<td>Improved fractional shortening by propanolol. No effect on βAR. Protection against toxic effects of catecholamines?</td>
</tr>
<tr>
<td>Yamazaki et al</td>
<td>1989</td>
<td>(25)</td>
<td>Rat AR 8 weeks</td>
<td>No treatment</td>
<td>Increased synthesis of βAR from week 1-4 returning to baseline by week 8</td>
</tr>
<tr>
<td>Yoshikawa et al</td>
<td>1995</td>
<td>(26)</td>
<td>Rabbit AR Acute (7 days)</td>
<td>Alacepril for 7 days (ACEI)</td>
<td>Acute decrease in myocardial βAR density and norepinephrine content, prevented by alacepril</td>
</tr>
<tr>
<td>Yoshikawa et al</td>
<td>1993</td>
<td>(27)</td>
<td>Rabbit AR 4 weeks</td>
<td>No treatment</td>
<td>Reduced βAR density and myocardial catecholamine content after 1 week, normalization after 4 weeks</td>
</tr>
</tbody>
</table>

Table 4: Previously published animal studies suggesting a role of the adrenergic system or beta-adrenergic blockade in aortic valve regurgitation. AR: aortic valve regurgitation; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; βAR: myocardial beta adrenergic receptors. ACEI: angiotensin converting enzyme inhibitor. 
<table>
<thead>
<tr>
<th>Author</th>
<th>Year published</th>
<th>Ref. number in text</th>
<th>Population studied</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guzzetti et al</td>
<td>1998</td>
<td>(9)</td>
<td>5 patients severe AR mean LVEF 49%, vs 14 with AS and normal LVEF</td>
<td>Myocardial biopsies before and 6 months after AVR. No medication.</td>
<td>Up regulation of □AR density 6 months post AVR vs pre surgical levels. □AR density less effected than in pressure overload pre surgery (aortic stenosis)</td>
</tr>
<tr>
<td>Dzimiri et al</td>
<td>1996</td>
<td>(6)</td>
<td>30 patients with volume overload (AR ± MR) and normal LVEF vs pressure overload (AS)</td>
<td>Myocardial biopsies during heart valve surgery. No medication</td>
<td>Decreased □AR density in volume overload patients. Volume overload decreased □AR density more than pressure overload</td>
</tr>
<tr>
<td>Hashimoto et al</td>
<td>1996</td>
<td>(10)</td>
<td>11 Women, Takayasu arteritis Moderate or severe AR</td>
<td>4 year follow-up 9 patients received beta-blockers</td>
<td>Beta blockers well tolerated and did not have negative effects on clinical status or LVEF or LV dilatation.</td>
</tr>
<tr>
<td>Moncada et al</td>
<td>2000</td>
<td>(16)</td>
<td>20 Women, Takayasu arteritis Moderate or severe AR</td>
<td>7 year follow-up. All 20 received vasodilators. 10 also received a beta blocker: Metoprolol (n=4) Acebutolol (n=3) Celiprolol (n=1) Arotinolol (n=2)</td>
<td>Beta blockers well tolerated and did not deteriorate clinical status nor LVEF. Beta blockers decreased LVH vs no beta-blockers</td>
</tr>
<tr>
<td>Matsuyama et al</td>
<td>2000</td>
<td>(14)</td>
<td>59 patients. AVR for severe symptomatic AR, normal LVEF</td>
<td>1 year follow up after AVR. ACEI+BB (n=12) ACEI (n=12) BB (n=8) No ACEI/BB (n=27)</td>
<td>More decrease of LV volume and LVH vs pre surgical values after 1 year in patients receiving BB vs other groups (ACEI or not)</td>
</tr>
</tbody>
</table>

Table 5: Published studies (humans) suggesting a role of adrenergic system or beta blockade in aortic regurgitation. AR: aortic regurgitation; MR: mitral valve regurgitation. LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; AS: aortic valve stenosis; AVR: aortic valve replacement surgery; □AR: beta adrenergic receptors; ACEI: angiotensin converting enzyme inhibitor; BB: beta blocker.
Fig. 1
Plante et al.
Fig 2
Plante et al.
Fig. 3
**Fig. 4**
Fig. 5

**G_{s\alpha}**

MRNA expression (AU)

Sham  |  AR  |  MET

PKA

MRNA expression (AU)

Sham  |  AR  |  MET

Fig. 5