

Title: Usefulness of carvedilol in the treatment of chronic aortic valve regurgitation

Short title: Carvedilol treatment for aortic valve regurgitation

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Abstract

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 beta blockade with metoprolol exerted beneficial effects on LV remodeling and survival. Despite the recent publication of promising human data beta blockade in chronic AR still remains controversial. More data is needed to support this potentially new treatment strategy. We hypothesized that carvedilol (CAR) might be another safe treatment option in chronic AR considering its combined beta and alpha 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/day 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. β 1 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 beta-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 beta blockers in chronic aortic valve regurgitation.

Key words:

aortic valve regurgitation

volume overload

left ventricular hypertrophy

beta blockers

Introduction:

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

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 due to methodological differences or small sample size (2-4). Based on the hypothesis that the adrenergic system is over-activated early in the course of chronic AR even before heart failure occurs, we have previously evaluated the effects of beta 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 al reported beneficial effects of beta blockers in a retrospective study of 756 patients (7). The use of beta blockers in chronic AR nevertheless remains controversial mostly in fear that drug-induced bradycardia may prolong diastole and consequently augment regurgitant time and volume. More data is needed to strengthen the hypothesis that beta blockade early in the course of chronic AR may be protective. We therefore designed the present study to evaluate another beta blocking agent: carvedilol. Considering its beta and alpha blocking properties, we hypothesized that it would improve hemodynamics and LV remodeling in rats with chronic severe AR and preserved ejection fraction.

Material and Methods

Animal model of aortic regurgitation

24 male Wistar rats (300-350g, Charles River, Qc, Canada) had severe AR induced by retrograde puncture of the aortic valve leaflets as previously described (8,9) and randomly divided in 2 groups (n=12/gr): 1: AR untreated and 2: AR treated with carvedilol 30 mg/kg/day orally. 20 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/day 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 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 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 sacrificed, 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.

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

Analysis of mRNA accumulation by quantitative RT-PCR

Tissues stored frozen in RNAlater (Ambion, Austin, TX) were homogenized in Trizol (Invitrogen, Burlington, ON, Canada) and quantitative RT-PCR was conducted on the appropriate tissue samples. QuantiTech Primers (Qiagen, Mississauga, ON, Canada)

used for this study are listed in Table S1 (supplemental material). Cyclophilin A was used as a control. The quantification of gene expression was based on the $-2\Delta\Delta C_t$ method. Results are expressed relative to the sham group mRNA levels which were arbitrarily fixed at 1. Natriuretic peptide type A (ANP) and B (BNP) expressions were evaluated considering their close relation to filling pressures and symptomatic heart failure. Pro-collagens 1 and 3 as well as fibronectin expressions were studied as key components of interstitial myocardial fibrosis. The expression of myocardial adrenoceptors β_1 , β_2 and α_1 were evaluated in the context of adrenergic blockade. The expression of key regulators of extracellular matrix (ECM) turnover (matrix metalloprotease 2 (MMP2) and tissue inhibitor of metalloprotease 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 beta 1 and 2 (TGF β_1 and 2) and connective tissue growth factor (CTGF) were also studied as they are closely related to collagen and fibronectin production by myocardial fibroblasts.

Staining for capillary density measurement

Sections of 8- μ m thickness were cut from the frozen left ventricle and were stained with isolectin B4 from *Bandeiraea simplicifolia* coupled with horseradish peroxidase (Sigma, Mississauga, Ont, Canada), and capillary density was analyzed in the subendocardial region of the LV myocardium (inner third). Pictures of three different LV fields of 8 animals per group were taken at 200X 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 (Carv) was significant. Student t test was used when two groups were compared directly. Statistical significance was set at p 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. 4 animals died in the non-treated AR group and 3 in the AR-carvedilol group. 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. Animal with AR (treated or not) developed severe cardiac hypertrophy as shown by their increased total heart weight but carvedilol treatment significantly reduced left ventricular, 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 to untreated AR. Ejection fraction was also improved as well as the myocardial performance index (MPI).

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 CAR in the AR group. The lower heart rate combined with an unchanged stroke volume resulted in a reduced calculated cardiac output in the CAR-AR compared to 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 + or - values. LVEDP was increased in the AR group and was normalized by carvedilol treatment.

Atrial (ANP) and brain (BNP) natriuretic peptides and Follistatin-like protein 1 (fstl1) mRNA expression

The relative expression of ANP, BNP and 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 over-expression 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.

Extracellular matrix (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 panel) was increased in untreated AR animals. Carvedilol significantly reduced this over-expression 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 (LOX1, MMP2, TIMP1, TGF β 1, TGF β 2 and CTGF) was evaluated. Results are summarized in figure 3. All expressions were increased in the AR groups compared to sham-controls. Carvedilol tended to reduce the overexpression of LOX1 and TIMP1 while there were no significant effects on the other factors.

Adrenergic receptors

The results of mRNA expression of adrenergic receptors β_1 , β_2 and α_1 in LV tissues are shown in figure 4. AR significantly reduced the mRNA expression of β_1 receptors in the LV tissue and normal levels were restored by carvedilol treatment. Although AR had no significant impact on β_2 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.

Capillaries 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 to 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 still 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 the fact 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 anymore the use of vasodilators to treat chronic AR with normal ejection fraction (1). An over-activation 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 al published in 2009 the results of a study of a retrospective cohort of 756 patients suggesting that beta blockers are beneficial for survival in patients with chronic AR (7). The attached editorial suggested that the recommendations not to use beta-blockers in AR patients should be removed from the treatment guidelines and that prospective large scale studies should be designed to test the effects of beta blockers in AR appropriately (16). Nevertheless fear still remains in the clinical world that beta 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 beta-blocker therapy after aortic valve replacement (17). The safety of beta-blockers in patients with AR from Takayasu arteritis has been reported in a few small Japanese studies in the past but clinical data about beta-blockers in AR remains very limited (18-20).

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 beta-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 of -20 bpm). This decrease in heart rate was not accompanied by an increase in AR severity or stroke volume. Although the bradycardia was modest, it was not deleterious and did not result in any negative hemodynamic impact. The rationale to choose carvedilol for this study was based on its proven effectiveness and good tolerance even in very sick patients with established systolic heart failure and on the pharmacological profile of the drug (21-23). On top of beta-blocking effects, carvedilol has been shown to have alpha-blocking properties which could theoretically be beneficial by reducing the afterload that is increased in chronic AR (24). High doses carvedilol also have theoretical anti-oxidant properties that may prove to be protective on a stressed LV myocardium. Our data confirms the β blocking effects (lower heart rate and restoration of β adrenoreceptor expression). The α blocking effects were not clearly demonstrated on measured hemodynamics (table 3) or α adrenoreceptor expression in the LV. However, direct measurements of peripheral α adrenoreceptor activity or peripheral resistance were not performed and small effects could have been missed. Peripheral α adrenoreceptor activity on explanted vessels has not been assessed and may have been positively affected by carvedilol. Considering however the lack of any measurable effects on systolic, diastolic and pulse pressures, the peripheral α blocking effect of carvedilol does not seem to be a major factor explaining the global response to the drug. However, all the pressure measurements were made on anesthetized animals. Whether measurable 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 follistatin-like protein 1 levels remained unaffected by carvedilol suggesting that the stress on the LV has not been completely overcome. This was expected since 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 related to myocardial remodeling. Collagen I and LOX1 as well as TIMP1 over-expressions 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 sub-endocardial ischemia. In another animal model of the disease, bradycardial pacing improved capillary density and myocardial performance (25). 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 still 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 beta blockers. Combined with the recent

publication by Sambat et al. strongly suggesting positive effects in humans, the fear of using beta-blockers in the context of AR may be unjustified. Prospective carefully designed clinical trials testing the effects of beta blockade in AR need to be done to assess this potential new treatment avenue in a disease currently lacking any proven effective medical treatment.

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Conflict of interest disclosures:

None to declare for all the co-authors.

References

1. Bonow RO, Carabello BA, Chatterjee K, de Leon AC Jr, Faxon DP, Freed MD, Gaasch WH, Lytle BW, Nishimura RA, O'Gara PT, O'Rourke RA, Otto CM, Shah PM, Shanewise JS, Smith SC Jr, Jacobs AK, Adams CD, Anderson JL, Antman EM, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Lytle BW, Nishimura R, Page RL, Riegel B. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease. *J Am Coll Cardiol* 2006;48: e1-e146.
2. Mahajerin A, Gurm HS, Tsai TT. Vasodilator therapy in patients with aortic insufficiency: a systematic review. *Am Heart J* 2007;153:454–461.
3. Scognamiglio R, Rahimtoola SH, Fasoli G. Nifedipine in asymptomatic patients with severe aortic regurgitation and normal left ventricular function. *N Engl J Med* 1994;131: 689–694.
4. Evangelista A, Tornos P, Sambola A. Long-term vasodilator therapy in patients with severe aortic regurgitation. *N Engl J Med* 2005;353:1342–1349.
5. Plante E, Lachance D, Gaudreau M, Drolet MC, Roussel E, Arsenault M, Couet J. Effectiveness of beta-blockade in experimental chronic aortic regurgitation. *Circulation* 2004;110 :1477-1483.
6. Plante E, Lachance D, Champetier S, Drolet MC, Roussel E, Couet J, Arsenault M. Benefits of long-term beta-blockade in experimental chronic aortic regurgitation. *Am J Physiol Heart Circ Physiol* 2008; 294:H1888-895.
7. Sampat U, Varadarajan P, Turk R Kamath A, Khandhar S, Pai RG. Effect of beta-blocker therapy on survival in patients with severe aortic regurgitation results from a cohort of 756 patients. *J Am Coll Cardiol* 2009;54: 452-457.

8. Plante E, Couet J, Gaudreau M, Dumas MP, Drolet MC, Arsenault M. Left ventricular response to sustained volume overload from chronic aortic valve regurgitation in rats. *J Card Fail* 2003;9: 128–140.
9. Arsenault M, Plante E, Drolet MC, Couet J. Experimental aortic regurgitation in rats under echocardiographic guidance. *J Heart Valve Dis* 2002;11: 128–134.
10. Anzai T, Yoshikawa T, Baba A, Nishimura H, Shiraki H, Nagami K, Suzuki M, Wainai Y, Ogawa S. Myocardial sympathetic denervation prevents chamber-specific alteration of beta-adrenergic transmembrane signaling in rabbits with heart failure. *J Am Coll Cardiol* 1996;28:1314–1322.
11. Guzzetti S, Mennini T, Cagnotto A, Di Biasi P, Scrofani R, Mezzetti S, Cogliati C, Paglia S, Malliani A. Myocardial beta-adrenergic and muscarinic receptor density in cardiac pressure or volume overload. *J Mol Cell Cardiol* 1998;30: 2095–2102.
12. Suzuki M, Yoshikawa T, Wainai Y, Nagami K, Handa S. Beta-blockade prevents ventricular failure following aortic regurgitation in rabbits. *Cardiology* 1997;88: 418–424.
13. Xiong L, Bouanani NE, Su JB, Crozatier B. Propranolol therapy in experimental heart failure in rabbits improves cardiac response to catecholamines without beta-adrenoceptor up-regulation. *Fundam Clin Pharmacol* 1995;9: 522–530.
14. Yamazaki T, Uematsu T, Mizuno A, Takiguchi Y, Nakashima M. Alterations of cardiac adrenoceptors and calcium channels subsequent to production of aortic insufficiency in rats. *Arch Int Pharmacodyn Ther* 1989;299: 155–168.
15. Yoshikawa T, Handa S, Yamada T, Wainai Y, Suzuki M, Nagami K, Tani M, Nakamura Y. Sequential changes in sympatho-neuronal regulation and contractile function following aortic regurgitation in rabbit heart. *Eur Heart J* 1993;14: 1404–1409.

16. Elkayam U. Beta-blockers in the treatment of aortic regurgitation a new opportunity? *J Am Coll Cardiol* 2009; 54:458-459.
17. Matsuyama K, Ueda Y, Ogino H. Beta-blocker therapy in patients after aortic valve replacement for aortic regurgitation, *Int J Cardiol* 2000;73:49–53
18. Hashimoto Y, Tanaka M, Hata A, Kakuta T, Maruyama Y, Numano F. Four years follow-up study in patients with Takayasu arteritis and severe aortic regurgitation; assessment by echocardiography. *Int J Cardiol* 1996;54: 173–S176.
19. Matsuyama K, Ueda Y, Ogino H, Sugita T, Sakakibara Y, Matsubayashi K, Nomoto T, Yoshimura S, Yoshioka T. Beta-blocker therapy in patients after aortic valve replacement for aortic regurgitation. *Int J Cardiol* 2000;73: 49–53.
20. Moncada GA, Hashimoto Y, Kobayashi Y, Maruyama Y, Numano F. Usefulness of beta blocker therapy in patients with Takayasu arteritis and moderate or severe aortic regurgitation. *Jpn Heart J* 2000;41: 325–337.
21. Krum H. Tolerability of carvedilol in heart failure: clinical trials experience. *Am J Cardiol* 2004; 93:58B-63B.
22. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. Effect of carvedilol on morbidity and mortality in chronic heart failure. *N Engl J Med* 1996; 334:1349–1355.
23. Poole-Wilson PA, Swedberg K, Cleland JGF, Di Lenarda A, Hanrath P, Komajda M, Lubsen J, Lutiger B, Metra M, Remme WJ, Torp-Pedersen C, Scherhag A, Skene A. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure. Results of the Carvedilol or Metoprolol European Trial. *Lancet* 2003; 362: 7-13.

24. Shannon R, Chaudhry M. Effect of alpha1-adrenergic receptors in cardiac pathophysiology. *Am Heart J* 2006;152: 842-850.
25. Wright AJ, Hudlicka O, Brown MD. Beneficial effect of chronic bradycardial pacing on capillary growth and heart performance in volume overload heart hypertrophy. *Circ Res* 1989;64: 1205–1212.

Figure legends

Figure 1:

mRNA expression of atrial natriuretic peptide (ANP-top panel), brain natriuretic peptide (BNP-middle panel) and follistatin-like protein 1 (fstl1- bottom panel). White columns: untreated groups; black columns: carvedilol groups. P values from 2-way ANOVA and Bonferroni post testing when applicable to evaluate disease, treatment or disease-treatment interactions (D X T) are shown to the right of each panel. *: $p < 0.05$ vs. corresponding untreated group.

Figure 2:

mRNA expression of collagen type 1 (Col I-top panel), collagen type III (Col III middle panel) and fibronectin (Fn- bottom panel). White columns: untreated groups; black columns: carvedilol groups. P values from 2-way ANOVA and Bonferroni post testing when applicable to evaluate disease, treatment or disease-treatment interactions (D X T) are shown to the right of each panel.

Figure 3:

mRNA expression of lysyl oxidase 1 (LOX1), matrix metalloprotease 2 (MMP2), tissue inhibitor of metalloprotease 1 (TIMP1), tissue growth factor beta 1 and 2 (TGF β 1 and 2) and connective tissue growth factor (CTGF). White columns: untreated groups; black columns: carvedilol groups. Solid line shows gene expression for untreated AR animals arbitrarily fixed at 1. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ vs. normal sham group.

Figure 4:

mRNA expression of adrenergic receptors (AR) beta 1, 2 and alpha 1 (β_1 , β_2 , α_1). White columns: untreated groups; black columns: carvedilol groups. P values from 2-way ANOVA and Bonferroni post testing when applicable to evaluate disease, treatment or disease-treatment interactions (D X T) are shown to the right of each panel. **: $p < 0.01$ vs. corresponding untreated group.

Figure 5:

Myocardial capillaries density. Typical examples are shown for normal sham and AR untreated rats. Bottom: quantification of myocardial capillaries density per field. White columns: untreated groups; black columns: carvedilol groups. P values from 2-way ANOVA and Bonferroni post testing when applicable to evaluate disease, treatment or disease-treatment interactions (D X T) are shown to the right of the bottom panel.

Table 1. Data at sacrifice.

Parameters	Sham (10/10)	Sham Carv (10/10)	AR (8/12)	AR Carv (9/12)	Disease (D) P value	Treatment (T) P value	D x T P value
Body weight, g	766 ± 10.3	771 ± 17.7	740 ± 11.7	758 ± 28.0	0.33	0.55	0.73
Tibial length, mm	65.8 ± 0.30	65.6 ± 0.47	64.4 ± 0.51	65.3 ± 0.63	0.11	0.49	0.29
Heart weight, mg	1476 ± 20.5	1508 ± 45.6	2661 ± 107.3	2190 ± 91.3***	<0.0001	0.006	0.0009
LV weight, mg	1023 ± 19.7	1028 ± 31.8	1881 ± 72.4	1598 ± 59.8**	<0.0001	0.020	0.016
RV weight, mg	301 ± 5.7	287 ± 6.0	459 ± 23.5	369 ± 19.2**	<0.0001	0.0059	0.039
Left atria weight, mg	33 ± 2.4	39 ± 4.3	93 ± 10.6	56.5 ± 4.3**	<0.0001	0.034	0.0037
Lung weight, g	2.6 ± 0.14	2.5 ± 0.16	3.4 ± 0.31	2.9 ± 0.25	0.046	0.36	0.43

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 X T) was found to have a P value below 0.05, a Bonferroni post-test was conducted: **: p<0.01 and ***: p<0.001 vs. untreated AR group. LV: left ventricle, RV: right ventricle.

Table 2. Echocardiography data

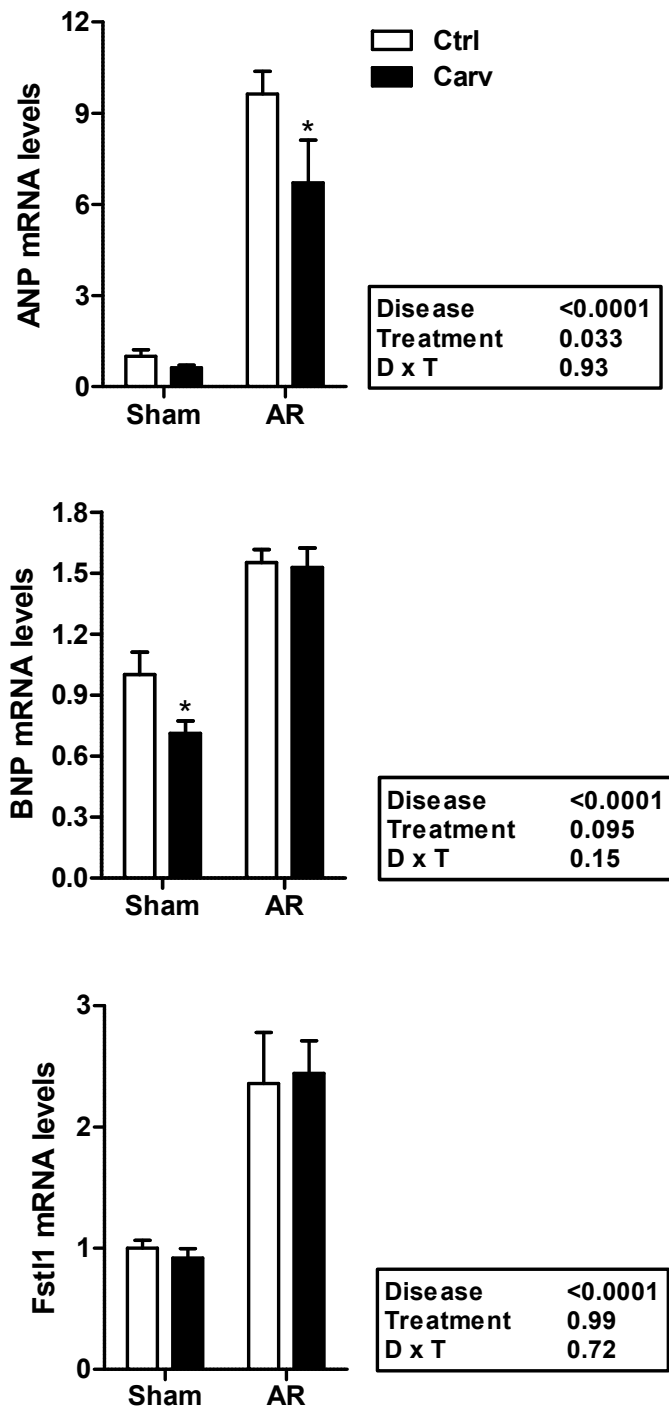
Parameters	Sham	Sham Carv	AR	AR Carv	Disease (D)	Treatment (T)	D x T
					P value	P value	P value
EDD, mm	9.2 ± 0.09	8.9 ± 0.15	12.4 ± 0.26	11.4 ± 0.37	<0.0001	0.015	0.23
ESD, mm	4.4 ± 0.10	4.4 ± 0.10	7.0 ± 0.14	6.1 ± 0.32*	<0.0001	0.0098	0.028
SW, mm	1.5 ± 0.03	1.6 ± 0.04	1.8 ± 0.02	1.6 ± 0.06*	0.0001	0.16	0.038
PW, mm	1.6 ± 0.04	1.6 ± 0.03	1.8 ± 0.03	1.8 ± 0.03	<0.0001	0.91	0.50
RWT (unitless)	0.34 ± 0.008	0.35 ± 0.006	0.29 ± 0.008	0.30 ± 0.008	<0.0001	0.10	0.71
EF, %	77 ± 0.8	76 ± 0.6	66 ± 0.5	71 ± 1.4**	<0.0001	0.036	0.0051
MPI (unitless)	0.42±0.018	0.41±0.007	0.52±0.032	0.45±0.011	0.0015	0.057	0.17
AR (% reg.)	na	na	78 ± 4.0	81 ± 4.0	na	na	na

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 X T) was found to have a P value below 0.05, a Bonferroni post-test was conducted: *: p<0.05 and **: p<0.01 vs. untreated AR group. EDD: end-diastolic diameter, ESD: end-systolic diameter, SW: septal wall thickness, PW: posterior wall thickness, RWT: relative wall thickness ((SW+PW)/EDD), EF: ejection fraction, MPI: myocardial performance index, AR: AR severity by echocardiographic semi-quantification. na: non applicable.

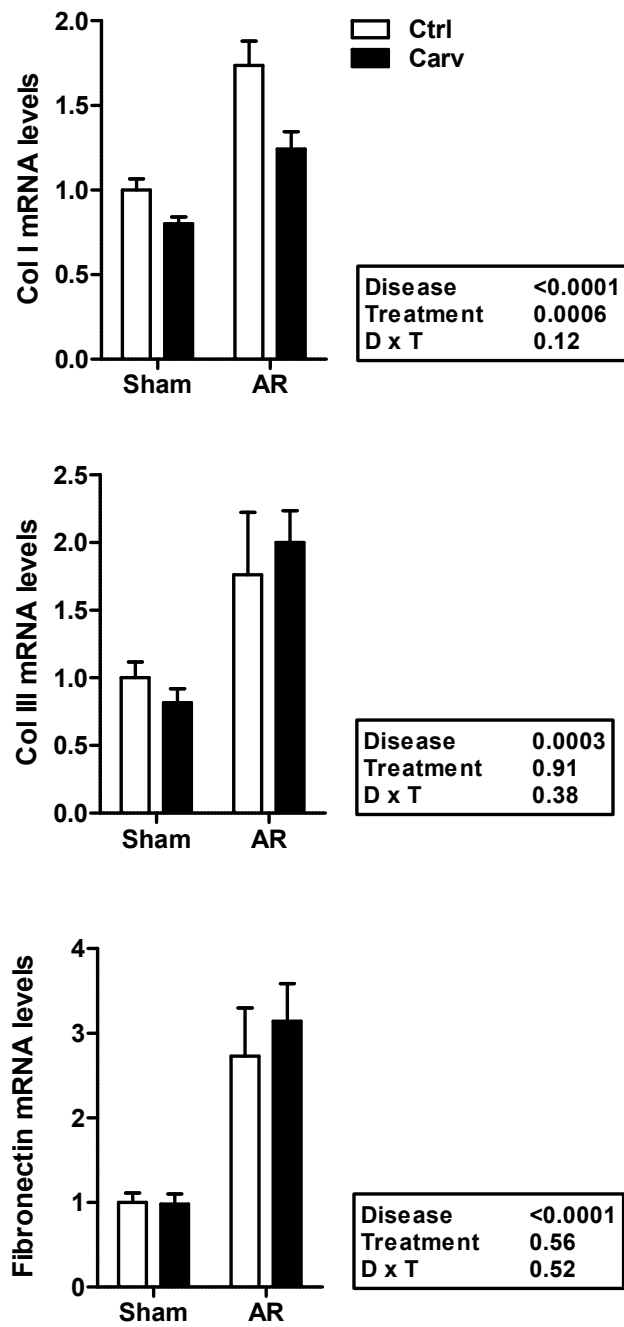
Table 3. Hemodynamics

Parameters	Sham	Sham Carv	AR	AR Carv	Disease (D)	Treatment (T)	D x T
					P value	P value	P value
HR (bpm)	361± 10.2	338 ± 9.9	352 ± 11.5	331 ± 10.7	0.48	0.040	0.93
SV (µl)	231 ± 4.7	224 ± 5.5	421 ± 16.7	381 ± 29.1	<0.0001	0.19	0.37
CO (ml/min)	83 ± 2.4	75 ± 2.9	147 ± 5.4	125 ± 8.4	<0.0001	0.012	0.21
SBP (mm Hg)	116 ± 2.2	123 ± 2.4	127 ± 4.5	130 ± 5.6	0.059	0.50	0.90
DBP (mm Hg)	88± 2.3	93 ± 2.2	63 ± 3.7	70 ± 4.4	<0.0001	0.27	0.59
dP/dt max	8580 ± 141.9	7753 ± 171.6	7047 ± 339.0	7711 ± 380.2	0.089	0.85	0.11
dP/dt min	7231 ± 287.7	6640 ± 201.7	4627 ± 183.1	4700 ± 170.1	<0.0001	0.88	0.18
EDP (mm Hg)	9 ± 0.3	9 ± 0.6	15 ± 1.0	10 ± 0.5**	0.0083	0.041	0.044

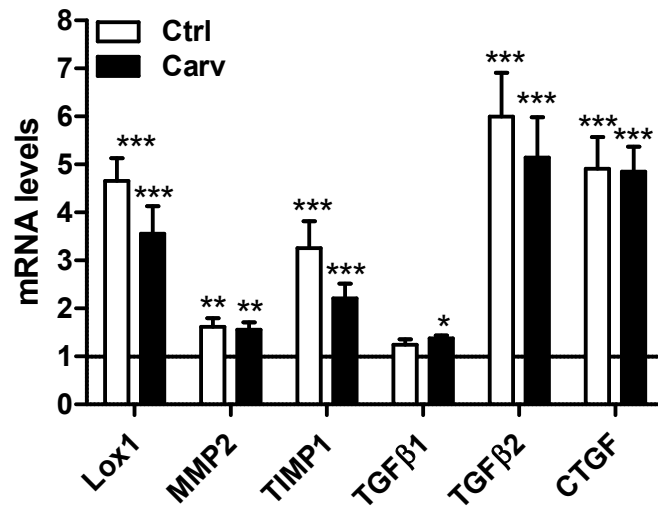
Values are expressed as mean ± SEM. P values from 2-way ANOVA analysis are shown on the right to evaluate separately the general impact of disease or treatment. If interaction between disease and treatment (D X T) was found to have a P value below 0.05, a Bonferroni post-test was conducted: **: p<0.01 vs. untreated AR group. 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. EDP: end diastolic pressure.



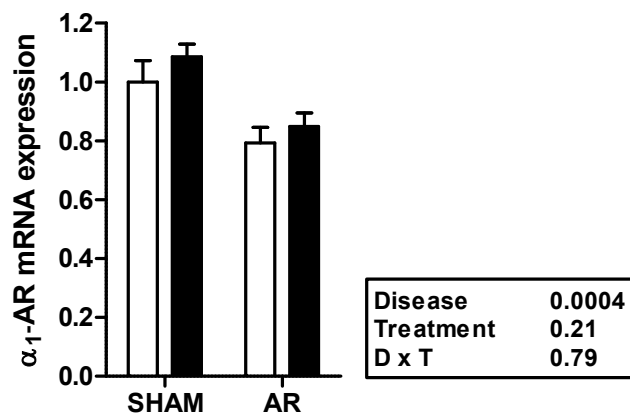
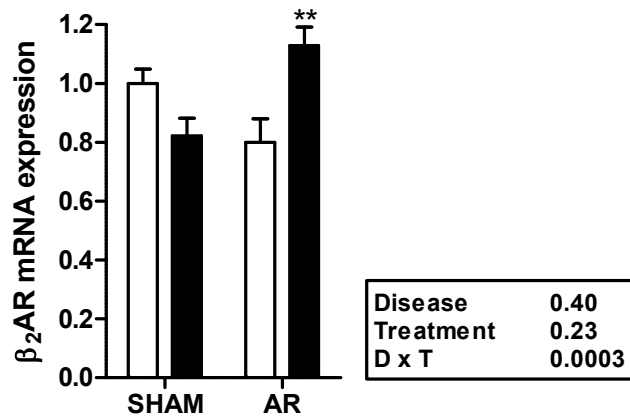
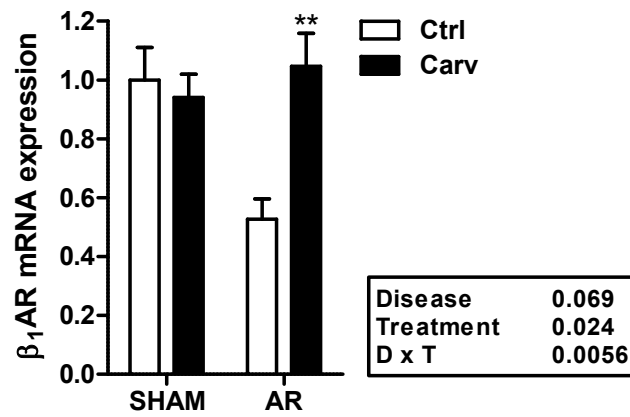
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Figure 1



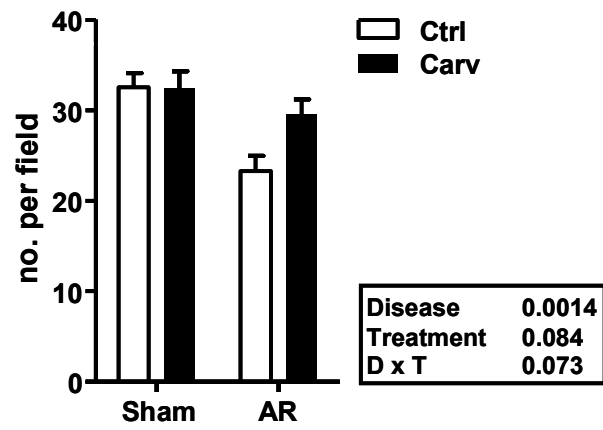
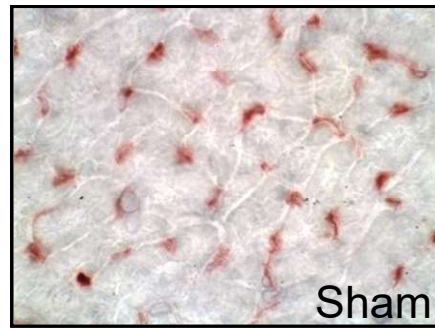
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Figure 2



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Figure 3



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Figure 4



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Figure 5