Title: A comparative study of vasodilators in an animal model of chronic volume overload caused by severe aortic regurgitation.

Eric Plante PhD, Dominic Lachance MSc, Jonathan Beaudoin MD, Serge Champetier PhD, Élise Roussel MSc, Marie Arsenault MD* and Jacques Couet PhD*.

Groupe de Recherche sur les Valvulopathies, Centre de Recherche Hôpital Laval, Institut de cardiologie de Québec, Université Laval, Quebec, Canada.

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*Corresponding authors: Marie Arsenault MD or Jacques Couet PhD

Centre de recherche de l'Hôpital Laval

2725 chemin Sainte-Foy,

Phone: 1-418-656-4510

Fax: 1-418-656-4544

Email: marie.arsenault@crhl.ulaval.ca

Abstract

Background: Aortic regurgitation (AR) is a disease of chronic left ventricular (LV) volume overload. Over time, AR will lead to LV dilatation, hypertrophy and loss of function. There is currently no medical treatment proven effective to slow the evolution of this cardiomyopathy. Vasodilators were once thought to have protective effects but recent publications have cast some doubts about their effectiveness. We hypothesized that drugs targeting the renin-angiotensin system (RAS) should be more effective than those having no direct effect on RAS.

Methods and Results: We designed a protocol comparing the effects of three vasodilators in a rat AR model (n=9-11 animals / group). The effects of a 6-month treatment of 1) nifedipine, 2) captopril or 3) losartan were compared in male AR rats. Sham-operated and untreated AR animals were used as controls. Nifedipine-treated animals displayed hemodynamics, LV dilatation, hypertrophy and loss of function similar to the untreated group. Both captopril and losartan were effective in improving hemodynamics, slow LV dilatation, hypertrophy and dysfunction. Gene expression analysis confirmed the lack of effects of the nifedipine treatment at the molecular level.

Conclusions: Using an animal model of severe AR, we found that vasodilators targeting RAS were effective to slow the development of LV remodeling and to preserve LV function. As recently shown in the most recent human clinical trial, nifedipine was totally ineffective. Targeting RAS seems a promising avenue in the treatment of this disease and clinical trials should be carefully designed to re-evaluate the effectiveness of ACEI or ARB in AR.

Key words:

aortic valve regurgitation

volume overload

vasodilators

renin-angiotensin system

Introduction:

Chronic volume overload such as seen in severe aortic regurgitation (AR) causes a progressive dilatation and hypertrophy of the left ventricle (LV). Paralleling this remodeling LV function eventually decreases, symptoms appear and valve replacement surgery often becomes necessary. Compared to other cardiac diseases, little is known about the treatment of chronic volume overload. In past decades several investigators have reported that medical therapy with vasodilators may be effective to reduce the aortic regurgitant volume and help maintain left ventricular function[1]. Nifedipine, a dihydropyridine calcium channel blocker, seemed to be an especially promising drug[2]. However, a more recent trial has failed to confirm any positive effects of nifedipine or enalapril treatment compared to placebo[3]. Considering these conflicting data, the AHA/ACC Valvular Heart Disease Treatment Guidelines no longer recommend any vasodilator for the medical management of chronic AR in patients with normal ventricular function[4]. In summary, no drug has yet been clearly shown to be able to slow LV dilatation, hypertrophy, or loss of systolic function or have any impact on morbidity or mortality in chronic AR[5]. Human clinical trials focusing on AR are unfortunately rare and usually include a limited number of patients with many confounding factors and pitfalls [5]. The study of an animal model of a disease can help overcome some of those pitfalls and offer the added benefit of providing cardiac tissues for analysis.

Our group has previously reported in an animal model of chronic AR that the renin-angiotensin system (RAS) is abnormally activated suggesting that blocking this system could to play an important role in preventing LV dilatation, hypertrophy and

loss of systolic function[6]. We have also shown in the same model that high doses of an angiotensin II converting enzyme (ACE) inhibitor such as captopril seem to be able to protect against AR cardiomyopathy both in normotensive as well as hypertensive rats[6;7]. Knowing that nifedipine does not target RAS, the current study was primarily designed to compare the effects of vasodilators targeting or not targeting RAS on the evolution of LV dilatation, hypertrophy and loss of systolic function. We hypothesized that RAS-targeting vasodilators would be the most effective.

Material and Methods

Animal model of aortic regurgitation

Sixty 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 5 groups (n=10-11/gr) as follows: 1) normal sham-operated animals (Sham); 2) AR untreated (AR); 3) AR+nifedipine (AR-N) (75mg/kg/d; po); 4) AR+captopril (AR-C) (1g/l in drinking water) or 5) AR+losartan (AR-L) (10mg/kg/d in drinking water). 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 treatments were started 2 weeks after the surgical procedure to allow for recovery from the acute phase and continued thereafter 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. LV were snap-frozen in liquid nitrogen and kept at -80^{o} 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 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. At

other times during the protocol, systolic and diastolic blood pressures were measured non-invasively using the tail-cuff method.

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 as previously described[10]. QuantiTech Primers (Qiagen, Mississauga, ON, Canada) used for this study are listed in Table 1. Cyclophilin A was used as a control. The quantification of gene expression was based on the $-2^{\Delta\Delta}$Ct method[11]. 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 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 (TGFβ1 and 2) and connective tissue growth factor (CTGF) were also studied as they are closely related to collagen and fibronectin production by myocardial fibroblasts.

Statistical analysis

Results are presented as mean ± SEM unless specified otherwise. Inter-group comparisons were done using one-way ANOVA and Tukey's post-test. 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).

Statement of responsibility

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Clinical data: All animals survived the duration of the protocol except for two in the nifedipine group. Both deaths were sudden, un-witnessed (occurred overnight) and were not accompanied by any symptoms or signs of impending heart failure in the preceding days. There were no signs of acute heart failure at necropsy in those two animals. Drugs were well tolerated. There was no clinical heart failure in any of the animals. Body weight was similar in all groups except for the captopril group in which the animals were smaller after 6 months (Table 2). Tibial length was also slightly shorter in the captopril group but this difference was minimal (less than 2%).

Tissue analysis:

Hearts were explanted at the end of the protocol and cardiac chambers were weighed. Results are summarized in table 2. As expected, untreated AR had severe LV hypertrophy as shown by the LV mass data reported in table 2. LV mass was similarly increased in the nifedipine group as the untreated group and was lower in captopril and losartan-treated animals. Right ventricular weight was increased in all AR groups compared to normal sham animals but less in the captopril group. Left atria were also larger in all AR groups. The largest left atria were found in the nifedipine group. Captopril and losartan had no impact on LA dilatation. Lung weights were similarly increased in all AR groups and drug treatment had no impact on that measurement.

Echocardiographic data: The data obtained after 6 months of treatment are summarized in Table 3. AR severity was similar in all AR groups. As expected, untreated AR animals (NT) developed severe LV diastolic and systolic dilatation and lower ejection fraction when compared to normal sham controls. Wall thickness remained similar in all groups. Relative wall thickness (RWT) was lower in untreated AR as expected in an eccentric pattern of LV remodeling. Results in the nifedipine group for LV dimensions, RWT and ejection fraction were similar to those of the untreated AR group. However, captopril and losartan significantly decreased the end-systolic dimensions. Ejection fraction was significantly better in the captopril and the losartan groups compared to the nifedipine group. RWT also tended to increase although this trend did not reach statistical significance. There was no significant difference between the results of the captopril and losartan groups.

Hemodynamic data: Hemodynamic data obtained after 6 months are summarized in table 4. Heart rate was similar in all groups. As expected in AR (volume overload), cardiac output was high in the untreated AR group compared to normal shams. Nifedipine did not decrease cardiac output. However, cardiac output was significantly lower and closer to normal in both captopril and losartan groups due to a smaller stroke volume. AR increased systolic and decreased diastolic blood pressure thereby increasing pulse pressure as expected. Both captopril and losartan decreased systolic blood pressure to a similar extent (mean of -12 mmHg and -8 mm Hg respectively). Nifedipine did not affect systolic blood pressure. Pulse

pressure in the nifedipine group remained high and similar to untreated AR animals whereas pulse pressure was the lowest in the captopril group. Losartan treatment decreased pulse pressure but to a lesser degree than captopril. Invasive intracardiac pressure measurements did not reveal any significant differences in dPdt+, dPdt- or LVEDP between any of the AR groups (results not shown).

ANP and BNP mRNA expression: The relative expression of ANP and BNP mRNA were measured after 6 months. Results are reported in figure 1. All AR groups displayed a significant increase in ANP mRNA expression as shown in the top panel or figure 1. Captopril (AR-C) treatment decreased this expression whereas the other treatments had no significant impact. BNP expression was significantly increased in untreated AR rats. Both losartan (AR-L) and captopril (AR-C) prevented this increase in BNP mRNA expression. However, nifedipine (AR-N) had no impact and animals treated with this drug had similar BNP expression as the untreated AR animals.

Extracellular matrix (ECM) remodeling-related mRNA expression:
Results for the mRNA relative expression of collagen I, collagen III and fibronectin are shown in Figure 2. Collagen I mRNA expression (top panel) was increased in untreated AR animals. Neither losartan nor nifedipine prevented this increase. Captopril strongly tended to normalize this parameter. Similar results were found for collagen III (middle panel). Captopril significantly prevented the increase of

collagen III mRNA. Fibronectin expression increased in all AR groups regardless of treatment (bottom panel).

Pro-MMP2 expression tended to increase in all AR groups but this trend did not reach statistical significance (figure 3, top panel). Treatments did not affect this parameter. TIMP-1 expression tended to increase in the untreated AR group but significantly increased in all 3 treatment groups (figure 3, middle panel). Losartan and captopril had a similar impact on TIMP-1 expression whereas the nifedipine group displayed the highest levels. Lysyl oxidase (LOX) expression (lower panel) was increased in all AR groups but mostly in the losartan and nifedipine group. LOX expression was similar in the untreated AR group and the one treated with captopril.

The level of expression of TGFβ1, TGFβ2 and CTGF were also evaluated (figure 4). The expression of TGFβ1 was increased in untreated AR animals (top panel). Only captopril could normalize this parameter. TGFβ2 expression was also increased in untreated AR (middle panel) and both losartan and captopril were able to decrease this over-expression. Nifedipine was ineffective on that aspect. Results similar to those of TGFβ2 were found for CTGF expression (bottom panel).

Discussion

Vasodilators such as nifedipine and ACEI have been the cornerstone of the pharmacological therapy for AR volume overload for many years[1]. Treatment of chronic volume overload remains however controversial and debated[12;13]. The available evidence suggests that this type of treatment may have some favorable effects but the limited evidence supporting or opposing the use of vasodilators in AR keeps us from drawing any firm conclusions[5;13].

In our study, we compared 3 vasodilators in a reproducible rat model of AR which is free of confounding factors or coexisting co-morbidities. We had previously reported that high doses of captopril were effective in rats with severe AR in both hypertensive and normotensive animals[6;7]. In the current study we conclude that nifedipine is not effective in our model as suggested by recent data in a human trial[3]. However, vasodilators targeting RAS definitively had some positive effects. Both losartan and captopril were able to slow LV hypertrophy and preserve LV ejection fraction. Both drugs effectively prevented the increase in cardiac output associated with volume overload. Captopril and Losartan also decreased systolic blood pressure as well as pulse pressure whereas nifedipine was unable to affect this parameter despite a high dosage. BNP expression was almost normalized by captopril and losartan whereas nifedipine had no effect at all on that parameter. Positive effects of the two RAS-targeting drugs were also found on the expression of fibrosis related molecules such as collagens I and III, LOX, TGFb1, TGFb2 and CTGF. These findings suggest that RAS-targeting drugs have protective effects on

the LV submitted to chronic volume overload before the occurrence of systolic heart failure.

All three drugs were given at or even a higher dosage that was previously proven to have effective antihypertensive effects[14-16]. It is interesting to note however that despite this high dose, nifedipine had no significant hemodynamic effect on the AR rats. On the opposite both captopril and losartan significantly reduced the cardiac output and decreased systolic pressure (similarly) and pulse pressure thereby decreasing the afterload. RAS inhibition therefore seems more effective to induce some hemodynamic benefits in our model of severe AR. It is also interesting to note that in the recent paper by Evangelista et al.[3] in which nifedipine and enalapril were found to have no positive effect on LV remodeling, the investigators reported that neither treatments (nifedipine 40 mg/day or enalapril 20 mg/day) had any hemodynamic effects on systolic or diastolic blood pressure.

The absence of effect on pulse pressure of high doses of nifedipine may seem intriguing. Captopril and losartan significantly reduced systolic blood pressure (and consequently pulse pressure) whereas nifedipine had no effect. Increased pulse pressure (with mild systolic hypertension) in AR is mostly related to mechanical causes increasing afterload: an increased ejection volume in a large arterial bed of fixed compliance. It is interesting to note that nifedipine treatment was unable to reduce the ejection volume whereas captopril and losartan had significant effects on that parameter. This lack of effect of nifedipine on the ejection volume may be one explanation for its lack of effect on systemic pressures. Secondly, part of the

relative hypertension in our model may be due to an increase in adrenergic drive and renin-angiotensin system activation as we have previously reported. Considering that nifedipine has no direct effect on the RAS and may cause an adrenergic hyper-activation, it seems logical that it was less effective to normalize pulse pressure. However this mechanism is probably less prominent in chronic AR since excessive small vessel vasoconstriction would have been expected to cause an increase (or at least stability) in diastolic BP whereas there is a significant decrease in diastolic BP in AR animals. This finding favors the mechanical hypothesis. We have to remember that pure chronic AR is not a “hypertensive” state and that it may not respond to anti-hypertensive treatments as expected. Captopril and losartan were both effective in our study whereas nifedipine was not. However, captopril and losartan did not yield totally similar results. They induced similar hemodynamic effects (comparable reduction in systolic blood pressure, normalization of stroke volume and cardiac output) but despite these similarities, captopril seemed to have additional benefits over losartan: captopril-treated animals had a slightly lower LV mass, smaller left atria, right ventricles and lungs as well as lower ANP expression. This suggests lower filling pressures in the captopril group. However, we were unable to detect any significant difference in LVEDP, dPdt- or echographic diastolic parameters to correlate with this hypothesis. It is possible that our study was underpowered to detect any significant difference in those measurements. It is also important to note that all measurements were done under anesthesia in a fasting state and that this might have affected invasive measurements and blunted small differences.

The LVs of the captopril-treated animals also displayed less collagen I, collagen III and LOX expression than the losartan group. These results suggest a less active extracellular matrix remodeling in the captopril group. Whether this would translate in added benefits versus treatment with losartan in the longer term remains to be established. Fibronectin mRNA expression on the other hand was not significantly modulated by either vasodilators used in this study. The accumulation of fibronectin in the LV of AR models has been described in the past[6;17-19]. We have previously shown that β-blockade could help normalize fibronectin expression. More importantly we have previously reported that captopril can reduce the total LV fibronectin content in AR rats[6;19;20]. The lack of effect of treatment on fibronectin mRNA expression in this protocol suggests that the turnover of fibronectin is still increased despite effective RAS blockade. Fibronectin has been shown to be regulated not only by RAS but also by stretch receptors. Considering that animals in the captopril and losartan groups had ejection volumes still more than 30% higher than normal, similar end-diastolic diameters and similar LVEDP than non-treated AR animals, we can suppose that stretch receptors in the LV were still significantly stimulated. Although collagen production by fibroblasts is also influenced by stretch receptors, it may be so in a lesser proportion.

In this study, we went a little further in describing the control of extracellular matrix remodeling. We observed the increase in mRNA levels encoding for the lysyl oxidase enzyme (LOX), an important player in the cross-linking of collagen fibers[21]. LOX has been shown to be up-regulated in rat models of LV hypertrophy as well as

in the heart of patients with congestive heart failure[22;23]. Here, we observed that captopril was able to abolish the up-regulation of LOX in the LV of AR rats which may help the LV maintain a better diastolic function[24]. This study also shows the implication of TGFβ signaling in the LV ECM remodeling. Again, targeting the RAS helped normalize the gene expression up-regulation of TGFβ1 and TGFβ2 as well as the one of CTGF. It is intriguing to observe this clear trend for enhanced collagen LV deposition which is not clearly correlated with increased amounts of collagen fibers[17;19]. We did observe increased peri-vascular collagen deposition in our rat AR model as well as increased general myocardial fibrosis after one year[10;25].

The differences between the captopril and losartan groups were not related to their hemodynamic effects since both drugs had similar impacts on hemodynamic parameters. Higher doses of losartan might have induced a more complete RAS blockade although the dose given to the animals in this protocol were already high and had measurable hemodynamic effects. It is known that RAS interacts with the sympathetic system at multiple levels and this interaction has been studied in heart failure models[26-28]. We have previously reported that the sympathetic system is over-activated in our model of chronic AR before heart failure occurs and that blocking this adrenergic over-activation is beneficial[10;19]. In a previous study by Balt et al[29], captopril was shown to be more effective than losartan to inhibit angiotensin II-induced facilitation of the sympathetic system despite maximal dosage of both drugs. In our study, animals in the captopril and losartan group had similar resting

heart rates (under anesthesia). However, we tested their heart rate response to a direct adrenergic stimulation (dobutamine infusion) and found that the heart rate increase was smaller in the captopril group compared to the losartan group (Capt. mean +53 bpm vs. Los +76 bpm) whereas both heart rate responses to dobutamine stimulation remained lower than the untreated AR group (mean +94 bpm in untreated AR). RAS-targeting drugs (captopril more than losartan) therefore seem to blunt the response to adrenergic stimulation in our model. The mechanisms of interaction between RAS and the adrenergic system in volume overload will be investigated more thoroughly in upcoming studies since the current protocol was not primarily designed to do so.

In conclusion, this study shows that captopril and losartan were effective to slow LV hypertrophy, remodeling and loss of ejection fraction in a model of chronic severe AR before the occurrence of heart failure. Captopril seemed to confer some advantages over losartan. Nifedipine was totally ineffective in this animal model, correlating with the most recently reported data in humans. Animal models obviously have their pitfalls and one must remain very cautious before extrapolating these results to humans. A more thorough evaluation of the adrenergic status of the animals with chronic severe AR as well as the impact of combination therapy such as the co-administration of captopril with a beta-blocker and/or losartan should be addressed in up-coming protocols and could yield important information. However, our findings suggest that nifedipine should

probably be discarded and that high doses of RAS-targeting drugs deserve to be adequately re-tested in carefully designed human AR clinical trials.

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Conflict of interest disclosures:

Eric Plante PhD : PhD studentship from the Canadian Institutes for Health Research

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Figure Legends

Figure 1: Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of the atrial and brain natriuretic peptide (ANP and BNP, respectively) after 6 months. Results are reported in arbitrary units (AU) as mean ± SEM (n=9-11/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR-NT: untreated; AR-L: losartan group; AR-C: captopril group; AR-N: nifedipine group. *: $p<0.05$ and **: $p<0.01$ vs. AR-NT group.

Figure 2: Gene expression of pro-collagens Type 1 and 3 and fibronectin in AR Wistar rats treated with different vasodilators for 6 months. Results are reported in arbitrary units (AU) as mean ± SEM (n=9-11/gr.). Sham group mRNA levels were normalized to 1. AR-NT: untreated; AR-L: losartan group; AR-C: captopril group; AR-N: nifedipine group. *: $p<0.05$ and **: $p<0.01$ vs. AR-NT group.

Figure 3: Evaluation by real-time quantitative RT-PCR of the LV mRNA levels for the pro-MMP2, TIMP1 and lysyl oxidase (LOX) in AR rats treated or not for 6 months. Results are reported in arbitrary units (AU) as mean ± SEM (n=9-11/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR-NT: untreated; AR-L: losartan group; AR-C: captopril group; AR-N: nifedipine group. *: $p<0.05$ vs. AR-NT group.

Figure 4: Gene expression of members of the pro-fibrotic TGFβ-CTGF signaling pathway is stimulated in the LV of AR rats. Results are reported in arbitrary units (AU) as mean ± SEM (n=9-11/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR-NT: untreated; AR-L: losartan group; AR-C: captopril group; AR-N: nifedipine group. *: $p<0.05$ and **: $p<0.01$ vs. AR-NT group.

Table 1: QuantiTect® Primer Assays used in Q-PCR analysis of gene expression.

mRNA	Symbol	Genbank Acc. No.	Amplicon size (bp)
Natriuretic peptide precursor type A	ANP	NM_012612	107
Natriuretic peptide precursor type B	BNP	NM_031545	94
Pro-collagen-1 alpha-1	Col1a1	NM_053304	92
Pro-collagen-3 alpha-1	Col3a1	NM_032085	111
Fibronectin	Fn	NM_019143	92
matrix metalloprotease 2	Mmp2	NM_031054	103
tissue inhibitor of metalloprotease 1	Timp1	NM_053819	113
Lysyl oxidase (LOX)	Lox	NM_017061	148
Transforming growth factor beta 1	Tgfb1	NM_021578	145
Transforming growth factor beta 2	Tgfb2	NM_031131	139
connective tissue growth factor	Ctgf	NM_022266	102
Cyclophilin A	Ppia	NM_017101	106

Table 2. Data at sacrifice (6 months).

Parameters	Sham	AR			
	(n=11)	NT (n=11)	Captopril (n=10)	Losartan (n=10)	Nifedipine (n=9)
Body weight, g	637 ± 17.5	687 ± 31.6	547 ± 16.0**	694 ± 14.9	653 ± 17.8
Tibial length, mm	59.7 ± 0.37	60.0 ± 0.32	58.7 ± 0.42*	59.7 ± 0.42	61.4 ± 0.22*
Heart weight, mg	1348 ± 37.1**	2217 ± 85.2	1713 ± 57.5*	1970 ± 72.2*	2260 ± 58.7
LV weight, mg	1000 ± 45.2**	1614 ± 65.5	1268 ± 56.2*	1382 ± 41.8*	1660 ± 46.8
RV weight, mg	288 ± 8.8**	364 ± 12.0	327 ± 13.9*	394 ± 21.1	382 ± 15.7
Left atria weight, mg	35.6 ± 3.67**	45.1 ± 2.27	44.1 ± 4.37	47.1 ± 4.04	56.5 ± 6.61*
Lungs weight, mg	19 ± 1.1*	23 ± 1.7	23 ± 2.3	26 ± 2.7	27 ± 3.6

Values are mean ± SEM. Indexation where indicated was made for tibial length. *: $p<0.05$ vs. AR-NT group and **: $p<0.01$ vs. AR-NT. LV: left ventricle, RV: right ventricle. NT: no treatment group.

Table 3. Echocardiography data (6 months)

Parameters	Sham	AR			
	(n=11)	NT (n=11)	Captopril (n=10)	Losartan (n=10)	Nifedipine (n=9)
EDD, mm	8.5 ± 0.23**	11.6 ± 0.32	10.8 ± 0.30	10.9 ± 0.17	11.8 ± 0.29
ESD, mm	4.3 ± 0.27**	7.7 ± 0.34	6.5 ± 0.39*	6.5 ± 0.22*	8.1 ± 0.37
SW, mm	1.9 ± 0.04	2.0 ± 0.05	2.1 ± 0.05	2.0 ± 0.05	2.0 ± 0.04
PW, mm	2.0 ± 0.02	2.1 ± 0.06	2.0 ± 0.05	2.1 ± 0.05	2.1 ± 0.03
RWT (unitless)	0.46 ± 0.016**	0.35 ± 0.013	0.38 ± 0.015	0.38 ± 0.008	0.35 ± 0.010
EF, %	74 ± 2.2**	55 ± 2.0	63 ± 2.7*	64 ± 2.1*	53 ± 2.6
MPI (unitless)	0.46±0.016**	0.35±0.013	0.38±0.015	0.38±0.008	0.35±0.010
AR (% reg.)	na	63 ± 4.4	64 ± 3.5	61 ± 3.2	60 ± 3.5

Echocardiographic measurements were made under 1.5% isoflurane anesthesia. Values are mean ± SEM. *: $p<0.05$ and **: $p<0.01$ vs. AR-NT 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 and AR: AR severity by semi-quantification. NT: no treatment group.

Table 4. Hemodynamics (6 months)

Parameters	Sham	AR			
	(n=11)	NT (n=11)	Captopril (n=10)	Losartan (n=10)	Nifedipine (n=9)
HR (bpm)	335 ± 12.0	341 ± 15.5	346 ± 13.0	342 ± 6.8	348 ± 10.3
SV (µl)	301 ± 12.7**	488 ± 24.4	395 ± 17.2*	401 ± 18.9*	495 ± 29.4
CO (ml/min)	101 ± 5.9**	163 ± 7.0	135 ± 4.7*	137 ± 6.8*	170 ± 5.8
SBP (mm Hg)	126 ±3.2	135±3.6	127±2.8*	123±1.8*	132±3.9
DBP (mm Hg)	79±3.2*	69±4.5	66±3.3	69±2.6	64±2.7

Measurements obtained under inhaled 1.5% isoflurane anesthesia. 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. Values are mean ± SEM. *: $p<0.05$ and **: $p<0.01$ vs. AR-NT group. NT. NT: no treatment group.

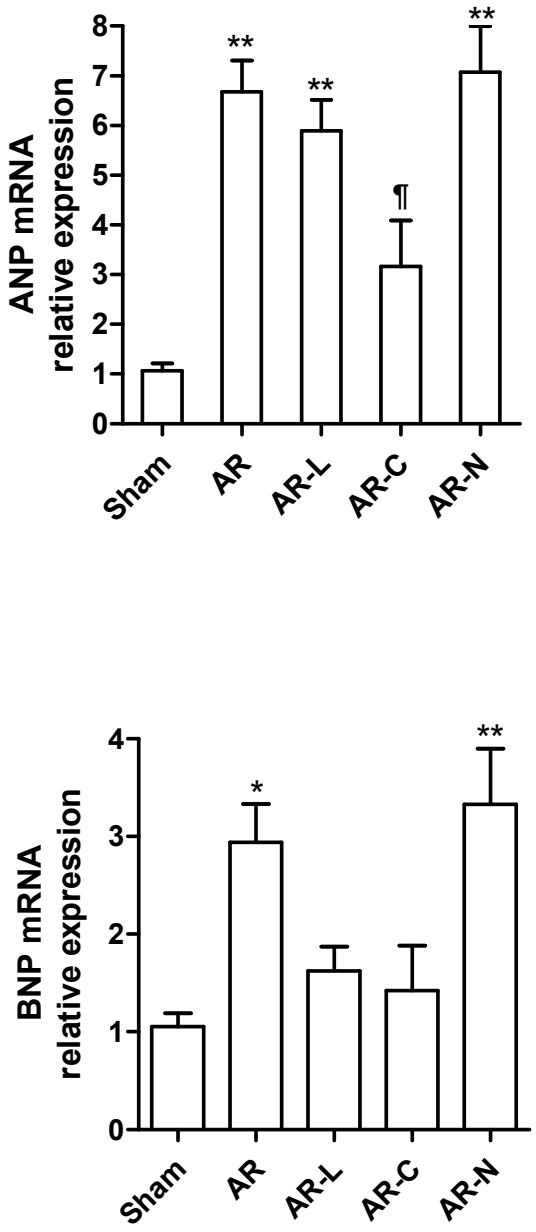

Fig. 1

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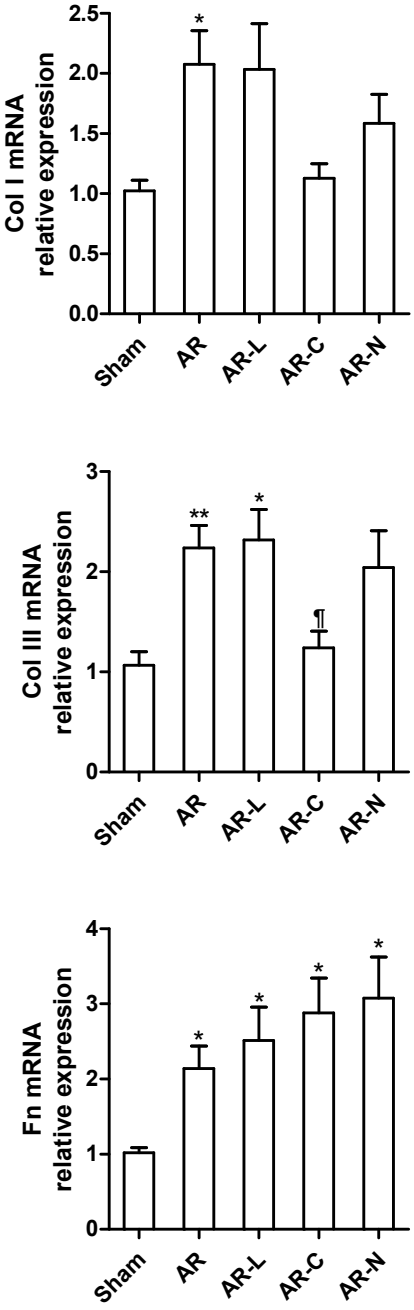

Fig 2

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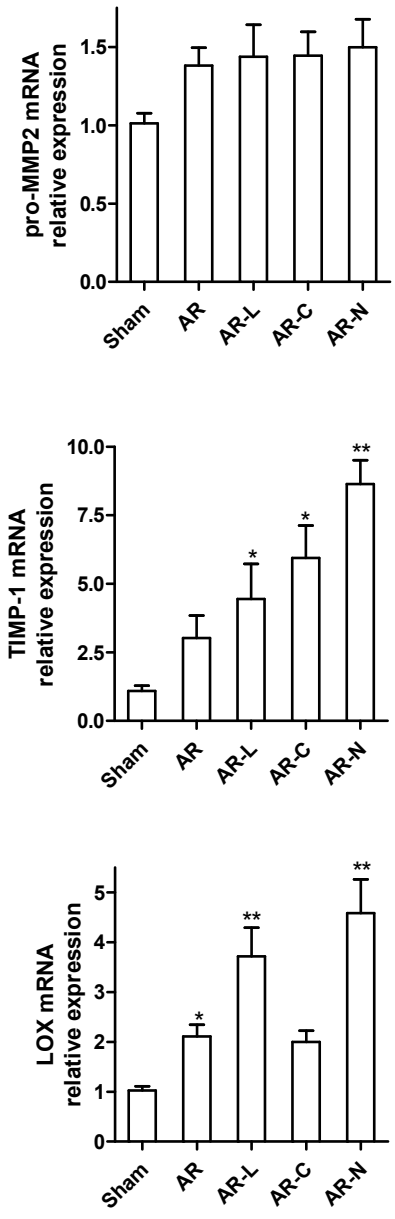

Fig. 3

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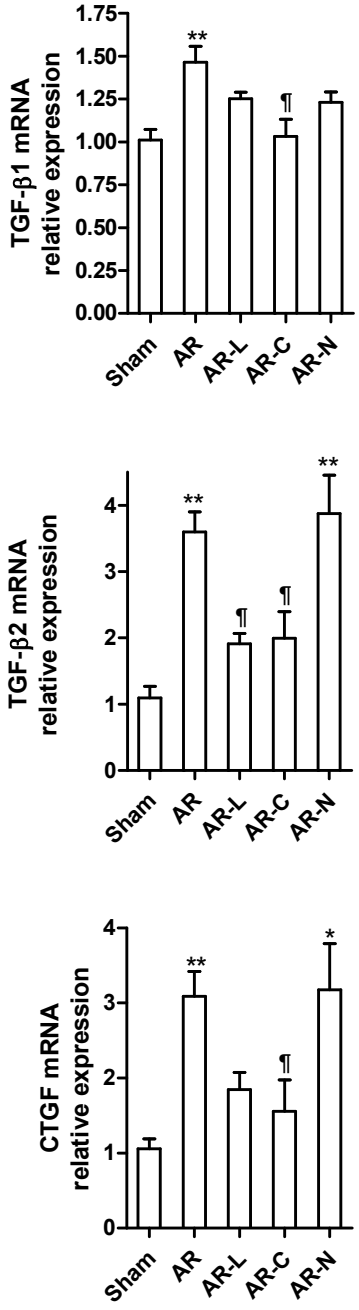

Fig. 4

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