Treatment of Combined Aortic Regurgitation and Systemic Hypertension: Insights From an Animal Model Study

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Background: Hypertension (HT) and aortic valve regurgitation (AR) often coexist but the specific impacts of AR + HT on the left ventricle (LV) are still unknown. The best treatment strategy for this combination of diseases is also unclear. The objectives of this study were 1) to evaluate LV function, remodeling and 2) to assess the effects of the angiotensin-converting enzyme (ACE) inhibitor captopril (C) in rats with AR + HT in spontaneously hypertensive rats (SHR).

Methods: Animals were grouped as follows: normotensive (NT) Wistar-Kyoto, NT + AR, hypertensive SHR (HT), and HT + AR receiving or not captopril (150 mg/kg/d). Hearts were evaluated in vivo by echocardiography and harvested for tissue analysis after 6 months of evolution.

Results: The HT + AR rats had the worst LV hypertrophy (LVH), subendocardial fibrosis, and lowest ejection fraction. Captopril normalized BP in HT and HT + AR, but could not prevent LVH in HT + AR as well as it did in isolated HT. The LV ejection fraction remained below normal in HT + AR + captopril compared to HT alone + captopril. Cardiomyocyte hypertrophy remained in HT + AR + captopril but was normalized in HT + captopril. Subendocardial fibrosis was reduced by captopril in HT + AR.

Conclusions: The AR + HT rats had the most severe myocardial abnormalities. High dose captopril was effective to slow LVH and preserve normal LV ejection fraction in isolated HT or AR, but was less effective when both pathologies were combined. Prohypertrophic stimuli clearly remain active in HT + AR despite ACE inhibition. These results suggest that a very aggressive medical treatment strategy may be required to optimize LV protection when AR and HT co-exist. Am J Hypertens 2006;19:843–850 © 2006 American Journal of Hypertension, Ltd.

Key Words: Hypertension, aortic valve regurgitation, rat, rennin-angiotensin system, captopril.
Methods

Animals

Sixty-six male Wistar-Kyoto (normotensive, NT) or SHR (hypertension [HT]) of 9 to 10 weeks of age (Harlan, Indianapolis, IN) were randomly divided into six groups (n = 10 to 14/group) as follows: 1) control sham-operated normotensive Wistar-Kyoto (NT); 2) normotensive with AR (NT-AR); 3) untreated sham-operated SHR (HT); 4) untreated HT-AR; 5) HT treated with captopril (HT-C); and 6) HT-AR treated with captopril (HT-AR-C). Captopril (150 mg/kg/d) (Sigma, Oakville Ontario, Canada) was given in drinking water.11–13 Drug treatment was started 2 weeks after the surgical procedure and continued for 24 weeks thereafter. This protocol was approved by the Université Laval’s animal protection committee.

Surgical Induction of Aortic Regurgitation

The AR was induced and graded in the animals as previously published, under ketamine/xylazine anesthesia, by retrograde puncture of one or two aortic valve leaflets.14–17 Only animals with severe AR by hemodynamic (acute decrease of diastolic BP >30 mm Hg) and echocardiographic criteria (by color and pulsed-wave Doppler as previously published (see next section)14–17 were included in the protocol. All animals were evaluated daily for the appearance of signs or symptoms of heart failure. Blood pressure was monitored measured by the tail-cuff method.

Echocardiography

A complete two-dimensional, M-mode, and Doppler echocardiogram was performed in ketamine/xylazine anesthetized animals, as previously published, at the following time points: preoperatively, immediately postoperatively, and before sacrifice time points: preoperatively, immediately postoperatively, and before sacrifice (24 weeks of treatment) using an Philips Sonos 5500 (Andover, MA) equipped with a 12-MHz probe.14,15,17

Tissue Analysis

At the time of sacrifice, the anesthetized rats were exsanguinated. The hearts were quickly removed, freed from connective tissue, and the left ventricle dissected and weighed. A piece of the left ventricle was immediately put in RNAguard (Ambion Inc., Austin, TX) solution and kept at −80°C until total RNA extraction. The remaining LV piece was minced, snap-frozen in liquid nitrogen, and then kept at −80°C.

Semiquantitative Reverse Transcription–Polymerase Chain Reaction of mRNA Accumulation

Reverse transcription–polymerase chain reaction (RT-PCR) analysis of mRNA accumulation of the different angiotensin II receptors subtypes such as collagen I, III, fibronectin, and pro-metalloprotease II was performed essentially as previously described.14,15

Cardiomyocyte Cross-Sectional Area and Fibrosis

The LV sections stained with trichrome–masson from at least 10 animals per group were analyzed as previously described.14,15 Results are expressed as cross-sectional area (CSA) mean ± SEM in arbitrary units. As for myocardial total and subendocardial (inner third of the LV wall) fibrosis, a ratio expressed as percentage of blue staining (collagen fibers) over total staining (red and blue) was estimated for each section using an image analysis software (SigmaScan, Systat Software, Inc., Point Richmond, CA).

Immunohistology

Angiotensin II-converting enzyme immunolabeling of LV sections was made using a purified anti-ACE mouse monoclonal antibody (MAB3502; Chemicon International, Temecula, CA) at 4 μg/mL. Analysis of the labeling was made by a blinded observer for the treatments on four LV sections per group, focusing on the subendocardial regions (inner third of the LV wall). Labeling was graded as negative (−), positive (+), or strongly positive (++) for each myocyte in the field. The percentage of each classification of myocyte labeling (−, +, or +++) per total myocyte number per field was evaluated and expressed as the mean ± SEM.

Statistical Analysis

Results are presented as mean ± SEM unless specified otherwise. One-way analysis of variance was performed to compare serial data. Statistical significance was set at P < .05 using post-hoc Tukey’s test. Data and statistical analysis were performed using GraphPad Prism version 3.02 for Windows, GraphPad Software (San Diego, CA).

Results

Part 1: Global Effects of the Combination of 24 Weeks of AR and HT on the Left Ventricle

Hemodynamics All SHR rats (HT) became severely hypertensive (Table 1). Severe AR resulted in a significant reduction of diastolic BP and in an expected increase in pulse pressure in both NT and HT groups. Stroke volume and cardiac output were increased in all NT-AR and HT-AR animals as expected in animals with severe AR.

Left Ventricular Hypertrophy and Dilatation The HT rats developed severe concentric LVH as shown by 1) the increased measured LV weights, 2) smaller intracavitary dimensions, 3) thicker LV walls, and 4) increased relative wall thickness (Tables 1 and 2). The NT-AR rats developed a more eccentric LVH as shown by the increased LV.
weight, intracavitary dimensions, and lower relative wall thickness (RWT). The HT-AR rats developed the most severe LVH compared with isolated AR or HT (Table 1).

Left Ventricular Systolic Function Table 2 summarizes the effects of AR on ejection fraction (EF) at the end of the protocol. The EF remained at more than 50% in all groups. The AR caused a significant decrease in EF in both NT and HT animals. The LV ejection fraction decreased the most from baseline in HT-AR rats (19.8%).

Left Ventricular Filling Parameters Filling parameters were evaluated on mitral outflow pulsed–Doppler recordings (Table 3). Left atrial dilatation and increased lung weight (indexed for body weight) were considered indirect signs of elevated left filling pressures. The E/A wave ratio remained unchanged in all groups. Mitral E wave slope was the steepest in HT-AR animals. The NT-AR and HT-AR rats had increased left atrial dimensions and lung weights in comparison with NT and HT animals without AR.

Cardiomyocyte Hypertrophy and Left Ventricular Subendocardial Fibrosis Myocyte CSA was significantly increased in the left ventricle of HT and HT-AR rats compared to NT. The AR alone in NT rats resulted in increased wall thickness (as shown in Table 2) but had limited effects on CSA. There was a trend toward higher values in HT-AR rats compared to NT and HT animals. The LV ejection fraction decreased the most in HT-AR rats (19.8%).

Part 2: Effects of Captopril Treatment

Hemodynamics Captopril treatment reduced significantly both systolic and diastolic BP in HT and HT-AR rats compared to untreated groups (P < .001) (Table 1). Pulse pressure remained above normal in HT-AR rats treated with captopril despite normalization of systolic BP. Cardiac output remained increased despite captopril treatment in AR and HT-AR groups.

Left Ventricular Remodeling and Function Captopril treatment had significant effects on LVH in HT-AR as well as in HT rats (Table 1, Figs. 2 and 3). The LV mass remained completely normal in HT animals treated by captopril. The LVH was partially prevented by captopril treatment in HT-AR but LV mass remained above normal in this group. Captopril completely prevented the concentric remodeling in HT (normal RWT). The RWT of treated and untreated HT-AR rats remained in a normal range. Captopril treatment slowed LV dilatation caused by the combination of AR and HT. Fig. 2 shows typical examples of the macroscopic appearance of the LV in each group.

The LV EF was lower in NT-AR and HT-AR groups compared to NT and HT as shown in Fig. 3 and the
relative decrease in EF was the most severe in HT-AR. This could not be prevented by captopril.

The increase in indexed lung weight observed in HT-AR rats was normalized by captopril. There was, however, no other significant effect of captopril on any of the measured Doppler diastolic parameters or on left atrial size (Table 3).

### Myocyte Cross-Sectional Area and Subendocardial Fibrosis
Captopril treatment significantly reduced myocyte CSA in the HT and HT-AR groups (Fig. 1). Myocyte CSA tended to remain higher than normal in the HT-AR treated with captopril but this result did not reach statistical significance (Figs. 1, 4, and 5).

In the animals not receiving captopril, the amount of subendocardial fibrosis in HT was higher than in NT. Captopril treatment had no significant effect on subendocardial fibrosis in the HT group, although a trend for lower levels seemed apparent. Captopril treatment reduced significantly the amount of subendocardial fibrosis in HT-AR rats but did not normalize this parameter when compared to controls.

Collagen I, collagen III, and fibronectin mRNA levels were significantly higher in the HT group and their levels were reduced by captopril treatment (Fig. 4). Captopril reduced collagen III and fibronectin expression in HT-AR but not the mRNA levels of collagen I. On the other hand, LV pro-MMP2 matrix metalloprotease 2 (MMP2) mRNA levels were lower in HT rats compared to NT rats.

### Tissue Renin-Angiotensin System
As illustrated in Fig. 5, LV ACE labeling in HT was increased compared to normal animals but was patchy (i.e., some myocytes labeled more than others), whereas in HT-AR animals the labeling was uniformly strong. Captopril treatment reduced ACE labeling in HT-AR rats. We did not observe any significant changes in the LV enzymatic ACE activity (not shown) as well as in the expression of angiotensin II receptors AT1a, AT1b, and AT2 mRNAs in any of the HT groups (± AR, ± C; results not shown). However, mRNA levels of the AT1a receptor were 45% lower in HT rats compared to NT rats (P < .05).

### Discussion
This study shows that a combination of AR and HT results in severe macroscopic and myocardial tissue abnormalities. Although ACE inhibition with high doses of captopril was very effective to prevent LVH and preserve normal EF in hypertensive animals, it was much less effective in the animals with a combination of AR and HT despite a complete normalization of their systolic BP.

Captopril was previously proven by other investigators to be effective in preventing and even inducing regression of LVH and remodeling very effectively in rats with HT (SHR) alone.2,12–14 Our team has also recently reported that a dose of 75 mg/kg/d effectively slowed LV dilatation and hypertrophy and preserved LV EF in animals with AR.14 Therefore, ACE inhibition has been proven effective for the treatment of AR or HT in rats when those pathologies occur separately. The ACE inhibitors have also been proven effective in humans with HT or aortic valve regurgitation.3,10 In the present study, high doses of captopril (150 mg/kg/d) given to animals with a combination of severe AR and HT slowed LVH and dilatation but was much less effective in maintaining normal EF than in animals with isolated AR or HT.

The normalization of systolic BP and the decrease in LVH in the HT-AR group treated with captopril is probably attributable to peripheral and tissue renin-angiotensin system inhibition, which resulted in afterload reduction. In animals with HT-AR, strong tissue ACE activation was present. However, hypertrophic stimuli clearly remained active despite ACE inhibition in the HT-AR rats. The mechanical workload induced by volume overload remained untouched by captopril, as shown by the increased stroke volume and cardiac output. Despite ACE inhibition, these ventricles remained exposed to a diastolic mechanical stretch that probably kept acting as a pro-hypertrophic stimulus.18–21 However, in a previous study in AR rats treated with captopril, we obtained better results on LV function, although the animals remained in a high output state and volume overload. The precise reason for the decreased effectiveness of ACE inhibition when AR and

### Table 2. Echocardiographical findings

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NT</th>
<th>AR</th>
<th>HT</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDD (mm)</td>
<td>8.7 ± 0.13</td>
<td>11.4 ± 0.29*</td>
<td>7.3 ± 0.18†</td>
<td>10.7 ± 0.13</td>
</tr>
<tr>
<td>∆EDD (mm)</td>
<td>0.31 ± 0.211</td>
<td>1.96 ± 0.391*</td>
<td>0.04 ± 0.217</td>
<td>3.92 ± 0.231*</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>4.7 ± 0.09</td>
<td>7.4 ± 0.38*</td>
<td>3.2 ± 0.13†</td>
<td>6.7 ± 0.16*</td>
</tr>
<tr>
<td>∆ESD (mm)</td>
<td>−0.20 ± 0.238</td>
<td>2.08 ± 0.436*</td>
<td>0.07 ± 0.136</td>
<td>3.71 ± 0.221*</td>
</tr>
<tr>
<td>SW (mm)</td>
<td>1.6 ± 0.04</td>
<td>1.8 ± 0.04</td>
<td>2.0 ± 0.03†</td>
<td>2.0 ± 0.04</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>1.6 ± 0.03</td>
<td>1.8 ± 0.04</td>
<td>2.2 ± 0.03†</td>
<td>2.0 ± 0.03</td>
</tr>
<tr>
<td>EF (%)</td>
<td>71 ± 1.5</td>
<td>58 ± 2.4*</td>
<td>80 ± 1.4†</td>
<td>60 ± 1.4*</td>
</tr>
</tbody>
</table>

AR = aortic regurgitation; EDD = end-diastolic diameter; EF = ejection fraction; ESD = end-systolic diameter; HT = hypertensive (SHR); NT = normotensive; PW = posterior wall thickness; Sham = sham-operated rats; SW = septal wall thickness. 

†P < .01 v corresponding sham group; † P < .01 v NT-Sham.

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HT are combined remains to be found. Aldosterone escape phenomenon has been reported despite treatment with captopril.\textsuperscript{22} It is possible that such a phenomenon may have occurred in the rats with a combination of HT and severe AR and contributed to the decreased effectiveness of captopril against LVH and fibrosis in this group. This hypothesis was not tested in our protocol but deserves further investigation.

The extracellular matrix (ECM) remodeling was also an important component in our HT-AR rats who developed severe subendocardial fibrosis. Although captopril seemed to partly prevent this increase in fibrosis in AR rats, the ECM was still abnormally rearranged.\textsuperscript{23,24} Captopril treatment did not normalize the expression of collagen I in HT-AR animals, whereas it had significant effects on collagen III and fibronectin. The reason for this remains unclear but despite this lack of effect on collagen I mRNA expression, captopril did have a significant effect on decreasing total fibrosis in HT-AR animals. This decrease in

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(Left) Left ventricular (LV) myocyte cross-sectional area (CSA) (top), LV total fibrosis (middle), and subendocardial fibrosis (bottom). Results are expressed in arbitrary units (CSA) or percent fibrosis as mean ± SEM (n = 10 animals per group). NT = normotensive; HT = hypertensive; AR = aortic regurgitation; UT = untreated; C = treated with captopril; Sham = sham-operated animals. *P < .05 and **P < .01 v corresponding untreated (UT) group, respectively. (Right) Typical views of trichrome-Masson stained subendocardial midventricular LV sections. Collagen fibers (blue); cardiomyocytes (red) (magnification x200).}
\end{figure}

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Parameters} & \textbf{Sham} & \textbf{AR} & \textbf{Untreated} & \textbf{Captopril} & \textbf{Untreated} & \textbf{Captopril} \\
\hline
Left atrial diameter (cm) & 0.39 ± 0.008 & 0.48 ± 0.012 & 0.46 ± 0.008\textsuperscript{*} & 0.44 ± 0.016 & 0.44 ± 0.016 & 0.43 ± 0.016 \\
Right atrial diameter (cm) & 0.29 ± 0.010 & 0.33 ± 0.010 & 0.35 ± 0.010 & 0.37 ± 0.010 & 0.37 ± 0.010 & 0.37 ± 0.010 \\
Ind. lung weight (mg/g) & 3.2 ± 0.000 & 3.3 ± 0.000 & 3.3 ± 0.000 & 3.3 ± 0.000 & 3.3 ± 0.000 & 3.3 ± 0.000 \\
E/A wave ratio & 2.0 ± 0.000 & 2.0 ± 0.000 & 2.0 ± 0.000 & 2.0 ± 0.000 & 2.0 ± 0.000 & 2.0 ± 0.000 \\
E wave downslope & 129.1 ± 45.2 & 135.1 ± 43.9 & 133.4 ± 43.9 & 133.4 ± 43.9 & 133.4 ± 43.9 & 133.4 ± 43.9 \\

\textsuperscript{*}P < .05 v corresponding sham group.
\end{tabular}
\caption{Atrial dimensions, lung weights, and Doppler LV filling parameters}
\end{table}
total fibrosis, despite a lack of effect on collagen I mRNA expression, could suggest an increased degradation of collagen I. However, pro-MMP2 mRNA expression was generally lower in HT rats, suggesting at least an abnormal degradation or turnover of the ECM. Borer et al. have reported in 2002 that fibronectin seems to play a more important role in AR-associated fibrosis than collagen I. Regulating mechanisms other than the renin-angiotensin system are probably involved in the production of collagen I in LVs submitted to severe overloading.

Experimental studies of models of combined pressure and volume overload of the left ventricle are rare. Most previous publications focused on overt heart failure by combining HT and arteriovenous fistulae or aortic banding and AR. These models quickly induce severe LV systolic dysfunction in the animals and are not relevant to the current study. Moreover, in those studies little tissue analysis was performed to assess the effects of combined overloads on the cardiomyocytes and on the ECM. Pharmacologic treatment, when given, was mostly limited to a few weeks’ duration and therefore the long-term effects of treatment have not been assessed.

Although LV remodeling and hypertrophy associated with isolated HT or isolated AR have been extensively studied, the management of patients with the combination of both LV overloads is not well established. Coexistence of HT and valvular regurgitation is not an uncommon situation. Based on previous publications, AR can be found in 6% to 8% of hypertensive patients and roughly
half of those patients have at least moderate AR. There is very little published data on this group of hypertensive patients. Recent data on a group of patients with HT and left-sided valvular regurgitation were published. Compared to subjects with similar levels of HT, patients with left-sided valvular regurgitation had significantly more macroscopic LV structural and functional changes. Even mild regurgitation had significant impacts in this cohort, namely larger LV internal dimensions and higher indexed LV mass. Similar findings were also described in a cohort of patients. The results from these two studies suggest that patients with a combination of HT and valvular regurgitation may need to be treated very aggressively. There are no specific recommendations for the pharmacologic management of patients with mixed pressure and volume overloads. The latest valvular heart disease treatment guidelines only suggest that hypertensive patients with significant AR should be treated with the aim of normalizing their BP as much as possible.

Study Limitations

Our findings definitely need to be assessed in human clinical trials before any hard conclusions can be drawn. The effects of milder degrees of AR in combination with HT remain to be explored as well as the potential effects of other types of medications, alone or in combination. The potential of reversibility of LVH and dilatation was not evaluated in our study. Longer follow-up studies as well as morbidity/mortality also need to be performed.

Conclusions

In our model, the combination of HT and AR had important impacts, not only on LV function and remodeling, but also on myocyte hypertrophy and the ECM, which were only partially prevented by ACE inhibition. In this animal model of AR + HT, reduction of afterload and ACE inhibition was not enough to protect the left ventricle. Normalization of systolic BP was not sufficient to protect the left ventricle of animals with combined overloads. Being well aware of the potential pitfalls of animal models, we do not suggest that the results of this study be transposed to humans. However, our results bring important insights on the response of the left ventricle to a combination of AR + HT and raise important questions on the optimization of the medical treatment of subjects with this combination of diseases.

References


