New insights in atrial remodeling
Jong, Anne-Margreet Rimke de
Angiotensin type 1 receptor blockade partially attenuates atrial remodeling due to ventricular pressure overload in mice

Anne Margreet De Jong, Isabelle C. Van Gelder, Laura M.G. Meems, Inge Vreeswijk-Baudoin, Silke U. Oberdorf-Maass, Alexander H. Maass

Submitted
Abstract

Aims: Cardiac diseases, such as hypertension or heart failure, are associated with atrial remodeling and increased occurrence of atrial fibrillation. We aimed to investigate the effects of ventricular pressure overload on atrial remodeling and the possible protective effects of the angiotensin II type 1 receptor blocker losartan.

Methods: Male mice were subjected to transverse aortic constriction (TAC) or sham operation with or without treatment with losartan (30 mg/kg/day), n = 6 per group. After five weeks echocardiographic and hemodynamic measurements were performed and animals were sacrificed. Atria were harvested and assayed for expression of genes associated with atrial remodeling.

Results: After five weeks of TAC, left ventricular (LV) systolic and diastolic function tended to be depressed and LV end-diastolic pressure (LVEDP) was increased. LV weight was increased. Ventricular pressure overload was associated with an increased atrial mRNA expression of brain natriuretic peptide (2.1-fold, median), skeletal α-actin, genes encoding collagens type I and III, profibrotic cytokines, such as connective tissue growth factor, and the proinflammatory cytokine interleukin-6 (3.5-fold, median). Losartan did not affect left ventricular and atrial weights but attenuated the effects of ventricular pressure overload on atrial mRNA expression levels for skeletal α-actin and regulator of calcineurin 1 (Rcan1), genes associated with hypertrophy. Expression levels of fibrotic and inflammatory markers were unaffected.

Conclusions: Ventricular pressure overload was associated with an increased mRNA expression of atrial genes associated with hypertrophy, fibrosis and inflammation. Losartan, instituted in the presence of persistent ventricular pressure overload, attenuated upregulation of genes associated with pathological hypertrophy.
Introduction

The most common cardiac arrhythmia atrial fibrillation (AF) is associated with atrial electrical and structural remodeling.\(^1\) Both are likely to contribute to the progressive nature of the arrhythmia.\(^1,3\) In animal models of AF structural changes include atrial dilatation, cellular hypertrophy, dedifferentiation, fibrosis, apoptosis, inflammation and myolysis.\(^4-6\) Risk factors for AF include hypertension and heart failure. Also these associated diseases cause atrial remodeling, as has been shown in heart failure models,\(^7,9\) in animal models of hypertension and ventricular pressure overload,\(^10-14\) as well as in patients with hypertension\(^15\) and mitral valve disease.\(^16\) Thus, atrial remodeling might already start long before AF occurs,\(^2\) as such creating a substrate for AF and making the atria more susceptible to develop AF. Although AF is often preceded by conditions associated with ventricular pressure overload, only limited studies assessed atrial structural remodeling in these conditions.\(^7,10,11,16\) We have observed atrial remodeling induced by transverse aortic constriction (TAC) and we found an association between the left ventricular end diastolic pressure (LVEDP) and the extent of atrial remodeling.\(^17\)

Treatment strategies aimed at preventing or delaying atrial remodeling might be beneficial in preventing AF occurrence or delaying AF progression. These treatments are called upstream therapies and target the atrial substrate. Treatments include blockade of the angiotensin II type 1 receptor blockers (ARBs), angiotensin-converting enzyme (ACE) blockers, statins and n-3 polyunsaturated fatty acids.\(^18,19\) Upstream therapy may improve long-term maintenance of sinus rhythm and atrial function. The mechanisms of potential beneficial effects, however, are not well documented. Only a few experimental studies assessed the effects of upstream therapy on atrial structural remodeling due to ventricular pressure overload.\(^12,13,20\)

In contrast to the promising effects of upstream therapy by ARBs in animal models of AF and heart failure,\(^8,21,22\) results in patients are less promising.\(^18,19,23-28\) This may relate to the late start of therapy in patients with a long history of AF and associated diseases which may have caused advanced remodeling. Therefore, it was our objective to investigate whether treatment with the ARB losartan affects atrial remodeling when started early in this process.

Methods

Animals

All experiments were approved by the local Committee on Animal Experimentation and were conducted under international guidelines on animal experimentation. 10-week old male C57Bl6/J mice were obtained from Harlan, The Netherlands. During the experiment, animals were kept on a 12 hr light:12 hr dark cycle with ad libitum access to food and water. This study was part from a larger study,\(^29\) from a random subset comprising of 6 mice per group, we harvested atria and conducted the analyses described in this paper.
Surgical procedures
Transverse aortic constriction (TAC) was performed as previously described.\textsuperscript{30} In short, mice were anesthetized using isoflurane (2% in O\textsubscript{2}), the thoracic cavity was opened between the 2nd and 3rd rib, and a blunted needle (27G) was placed on the aortic arch between both carotid arteries. Subsequently, the aorta was tied onto the needle with a 7.0 silk suture. Immediately thereafter the needle was removed, producing a reproducible stenosis of the aorta. Sham operations were performed in the same way, but the suture was removed instead of being knotted. Analgesic medication was administered for 48 hr from the start of the operation (Carprofen, 5 mg/kg subcutaneously). Losartan was dissolved in the drinking water, and the final dosage was 30 mg/kg/day, based on a previous study in our lab.\textsuperscript{31} Losartan was administered from the moment of surgery continuously until sacrificing.

Echocardiographic and hemodynamic measurements
Five weeks after TAC or sham operation, cardiac dimensions were measured using transthoracic echocardiography with a 14 MHz transducer (Vivid 7, GE Healthcare, Diegem, Belgium) as previously described.\textsuperscript{32} Mice were anesthetized using isoflurane (2% in O\textsubscript{2}) and the body temperature was maintained by placing the mouse on a heating pad. Prior to sacrifice hemodynamic measurements were obtained, using a Millar catheter (Mikro-tip 1.4F; SPR-839, Millar Instruments, Houston, TX, USA). Mice were anaesthetized as described above, and a pressure transducer catheter was inserted via the right carotid artery into the aorta, and thereafter into the left ventricle (LV). After the hemodynamic measurements, blood was taken via cardiac puncture and the heart was excised. Ventricles and atria were weighed separately. The atria were snap-frozen for RNA analysis.

Real-time quantitative PCR
Total RNA was isolated from whole atria using TRIzol reagent (Invitrogen Corporation, Breda, The Netherlands). cDNA was synthesized by QuantiTect Reverse Transcription kit (Qiagen, Venlo, The Netherlands) according to the protocol. RNA was isolated from the left and right atrium together. Gene expression levels were determined with Absolute QPCR SYBR Green ROX Mix (Abgene, Epsom, United Kingdom) in the presence of 7.5 ng cDNA and 200 nM forward and reverse primers. The Biorad CFX384 (Biorad, Veenendaal, The Netherlands) was used to do the qRT-PCR. The protocol used was as follows: initial denaturation and activation of the DNA polymerase at 95 °C for 3 min, followed by 35 cycles with denaturation for 15 s at 95 °C and annealing and elongation for 30 s at 60 °C and a melt curve. Gene expression levels were corrected for ribosomal protein, large, P0 (36b4) reference gene expression, and values were expressed relative to the control group. Primers used are shown in Table 1. The stretch-regulated stress markers atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured as markers of stretch, as a surrogate for pathological hypertrophy and as markers for the severity of the heart disease. Skeletal α-actin was investigated as a marker of hypertrophy and dedifferentiation, and regulator of calcineurin 1 (Rcan1) was used as a marker of calcineurin activity. Genes associated with fibrosis were
also investigated: transforming growth factor β1 (TGFβ1) and connective tissue growth factor (CTGF) are profibrotic growth factors, collagen type I and type III encode the major collagens in the heart, fibronectin is a glycoprotein of the extracellular matrix. In the heart periostin plays a role during development and in remodeling. Matrix metalloproteinase 2 (MMP2) and tissue inhibitor of metalloproteinases 1 (TIMP1) are involved in the dynamics of extracellular matrix. The inflammatory cytokines interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) were chosen as they have been shown to be related to AF, in addition, the expression of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) was measured. To investigate endothelial function, which has been associated with AF, we used expression of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), vascular cell adhesion molecule 1 (VCAM) and vascular endothelial growth factor A (VEGFa).

Table 1: Primers

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Reverse</th>
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<td>gcaggcgcgaatgcagatgg</td>
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<tr>
<td>ACTA1</td>
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</tr>
<tr>
<td>ANP</td>
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<td>tctaccgcactttctctc</td>
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<tr>
<td>BNP</td>
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<td>ggaagagaccaggagaga</td>
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<td>Collagen type III</td>
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<td>cccagttctaatgtcaca</td>
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<td>ctgggtggttttgatagtc</td>
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<tr>
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<td>eNOS</td>
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<td>Fibronectin</td>
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<td>iNOS</td>
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</tr>
<tr>
<td>Rcan1</td>
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<td>MCP-1</td>
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<td>ggagctgtgctgtgtagttag</td>
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<td>Periostin</td>
<td>gatcaccagggacagctcc</td>
<td>cccacccctctgtggaatcc</td>
</tr>
<tr>
<td>RANTES</td>
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<tr>
<td>TGFβ1</td>
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<td>TIMP1</td>
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</tr>
<tr>
<td>VEGFa</td>
<td>actgagcccttgcccttactg</td>
<td>cagtagtcctgctggtagac</td>
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ACTA1, skeletal α-actin; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CTGF, connective tissue growth factor; eNOS, endothelial nitric oxide synthase; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; Rcan1, regulator of calcineurin 1; MCP-1, monocyte chemotactic protein-1; MMP2, matrix metalloproteinase; RANTES, regulated on activation, normal T cell expressed and secreted; TGFβ1, transforming growth factor β1; TIMP1, tissue inhibitor of metalloproteinases 1; VCAM1, vascular cell adhesion molecule 1 and VEGFα, vascular endothelial growth factor A.
Chapter 4

Statistics
Data are expressed as median with 25th and 75th quartile. Comparisons between groups were done using the Kruskal-Wallis H test, followed by the Mann-Whitney U test. Non-parametric test were chosen to reduce the influence of the high variance. The order of the values is used to test for significance instead of the actual values. All analyses were done using IBM SPSS Statistics (Version 20, SPSS Inc., Chicago, IL, USA). P-values of <0.05 were considered statistically significant.

Results

Effects of ventricular pressure overload

Ventricular pressure overload and cardiac function

TAC resulted in an increased maximum aortic pressure (Table 2) and an increased left ventricular weight (Fig. 1a). Echocardiographic measurements showed an increased interventricular septum (IVS) and left ventricular posterior wall (LVPW) thickness, and a reduced fractional shortening in mice subjected to TAC (Table 2). Hemodynamic measurements revealed an increased left ventricular end-diastolic pressure (LVEDP), a reduced relaxation and non-significantly reduced contractility (Table 2).

Table 2: Echocardiographic and hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC + losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max pressure aorta (mmHg)</td>
<td>97.97 (96.85-98.94)</td>
<td>149.82 (112.28-160.11)**</td>
<td>135.83 (129.23-154.52)**</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.63 (0.58-0.66)</td>
<td>0.94 (0.87-1.13)**</td>
<td>0.91 (0.88-0.92)**</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.67 (0.64-0.73)</td>
<td>0.93 (0.89-1.05)**</td>
<td>0.91 (0.85-0.94)**</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.81 (3.45-4.02)</td>
<td>4.32 (4.08-4.63)*</td>
<td>4.40 (4.30-4.68)**</td>
</tr>
<tr>
<td>FS (%)</td>
<td>47.70 (44.03-49.00)</td>
<td>25.50 (21.28-28.88)**</td>
<td>32.9 (30.20-35.98)**###</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.65 (1.25-6.15)</td>
<td>16.86 (16.66;)*</td>
<td>8.10 (3.66-14.40)</td>
</tr>
<tr>
<td>dPdt max (mmHg/s)</td>
<td>7842 (6911-8232)</td>
<td>5755 (5742;*)</td>
<td>7152 (6885-7876)</td>
</tr>
<tr>
<td>dPdt min (mmHg/s)</td>
<td>-6277 (-7575-5800)</td>
<td>-4569 (-5157;)*</td>
<td>-5312 (-6354-5115)*</td>
</tr>
</tbody>
</table>

Results are expressed as median (25th ; 75th quartile). Echocardiographic measurements (n = 6 per group), hemodynamic measurements (n = 3-6 per group). * P < 0.05, ** P < 0.01 vs sham. * P < 0.05, ** P < 0.01 vs TAC. LVEDP, left ventricular enddiastic pressure; IVSd, diastolic interventricular septum; LVPWd, diastolic left ventricular posterior wall; FS, fractional shortening.
Figure 1: The effect of ventricular pressure overload on LV and atrial weight and on gene expression of genes associated with hemodynamic changes, hypertrophy and calcineurin signaling, in the atria of mice, 5 weeks after sham or TAC surgery. (A) LV weight, (B) atrial weight, (C) ANP, (D) BNP, (E) ACTA1 and (F) Rcan1. Results are expressed as median with interquartile range. * P < 0.05, ** P < 0.01, *** P < 0.001. LV, left ventricle; TL, tibia length; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ACTA1, skeletal α-actin; Rcan1, regulator of calcineurin 1. n = 6 per group.
Atrial remodeling

Atrial hypertrophy was suggested by an increased atrial weight after five weeks of pressure overload (Fig. 1b). The expression of genes in the atria associated with stretch and hypertrophy was increased: a non-significant increase of 1.3-fold of ANP expression (Fig. 1c) and a significant increased expression of BNP by 2.1-fold (Fig. 1d) were observed. The expression of skeletal α-actin was increased upon TAC (Fig. 1e), albeit with a high variation (median 24.5; range 0.9 – 1107, p < 0.05). The increased expression of skeletal α-actin suggests, besides pathological hypertrophy, also dedifferentiation. There was a non-significant increased expression of 2.1-fold of Rcan1, a marker for increased calcineurin activity (Fig. 1f).

Increased expression of genes associated with fibrosis was also observed (Fig. 2). The expression of the profibrotic cytokine CTGF (Fig. 2a) was increased. In addition, the expression of the two major collagen encoding genes, collagen type I (Fig. 2c) and type III (Fig. 2d), as well as of the extracellular matrix molecules fibronectin (Fig. 2e) and periostin (Fig. 2f), and two of the extracellular matrix regulatory proteins, MMP2 (Fig. 2g) and TIMP1 (Fig. 2h) were increased.

Inflammation was suggested by an increased atrial expression of IL-6 (Fig. 3a). The atrial expression of other inflammatory markers, MCP-1 (Fig. 3b) and RANTES (Fig. 3c), was unchanged. Assessment of changes in gene expression related to endothelial function did not reveal any effect of TAC, nor in eNOS, VCAM or in VEGF expression levels (Table 3).

Table 3: Relative atrial gene expression of genes associated with endothelial function

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC + losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS/36b4</td>
<td>0.85 (0.76-1.19)</td>
<td>0.81 (0.75-1.24)</td>
<td>1.03 (0.76-1.38)</td>
</tr>
<tr>
<td>VCAM/36b4</td>
<td>0.99 (0.86-1.19)</td>
<td>0.94 (0.81-1.11)</td>
<td>1.15 (0.95-1.34)</td>
</tr>
<tr>
<td>VEGFa/36b4</td>
<td>1.00 (0.94-1.09)</td>
<td>1.15 (0.84-1.36)</td>
<td>1.07 (0.99-1.17)</td>
</tr>
<tr>
<td>iNOS/36b4</td>
<td>1.00 (0.85-1.19)</td>
<td>1.31 (1.04-2.21)</td>
<td>1.68 (1.38-2.17)</td>
</tr>
</tbody>
</table>

Results are expressed as median (25th; 75th quartile). ## P < 0.01 vs TAC. eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; VCAM1, vascular cell adhesion molecule 1; VEGFa, vascular endothelial growth factor A. n = 5-6 per group.
Losartan partially reduces atrial remodeling.

Figure 2: Relative atrial gene expression of genes associated with fibrosis, 5 weeks after sham or TAC surgery. (A) TGFβ1, (B) CTGF, (C) collagen type I, (D) collagen type III (E) fibronectin, (F) periostin, (G) MMP2 and (H) TIMP1. Results are expressed as median with interquartile range. * P < 0.05, ** P < 0.01, *** P < 0.001. TGFβ1, transforming growth factor β1; CTGF, connective tissue growth factor; MMP2, matrix metalloproteinase 2; TIMP1, tissue inhibitor of metalloproteinases 1. n = 6 per group.
To investigate the possible beneficial effects of blockade of the angiotensin II type 1 receptor we assessed the effects of the ARB losartan in the presence of persistent ventricular pressure overload. The maximum pressure was increased to a similar level in both groups of mice subjected to TAC (Table 2), indicating that there were no differences between the ventricular loading conditions between the ARB treated and untreated mice. Losartan also did not affect the echocardiographic measurements, except for a slight but significant difference in fractional shortening (Table 2). The hemodynamic measurements showed a non-significantly reduced LVEDP and non-significant improvement in dP/dt max and dP/dt min (Table 2) in the mice treated with losartan. No effect of losartan treatment on heart rate was observed. LV weight was unchanged in the losartan treated mice (Fig. 1a).

On the atrial level, losartan had no effect on the TAC induced atrial hypertrophy (Fig. 1b) nor on ANP or BNP expression (Fig. 1c,d). In contrast, expression of skeletal α-actin, being a marker for hypertrophy and dedifferentiation, was significantly reduced (Fig. 1e). A possible mechanism via which losartan can exert beneficial effects, is via inhibition of calcineurin-
Losartan partially reduces atrial remodeling

related cell signaling. Even though Rcan1 levels, as a surrogate for calcineurin activity, were not significantly increased by TAC, losartan significantly decreased atrial Rcan1 mRNA levels when compared with mRNA levels in mice subjected to TAC without treatment (Fig. 1f).

Losartan did not significantly reduce expression of the fibrotic genes, except for TGFβ1. Expression of genes related to inflammation was unchanged in mice treated with losartan (Fig. 2). iNOS expression was significantly increased (1.7-fold) in animals subjected to pressure overload treated with losartan.

Discussion

In the present study the effects of permanent ventricular pressure overload by TAC on atrial remodeling were investigated. In addition, the effects of the ARB losartan in the continuous presence of ventricular pressure overload by TAC were studied. The main findings of the present study were: (1) ventricular pressure overload in this model impaired cardiac function, induced ventricular hypertrophy and changes in mRNA expression in the atria suggesting atrial remodeling, including hypertrophy, fibrosis and inflammation; (2) no changes in gene expression related to endothelial function were observed; (3) losartan slightly improved hemodynamic parameters, and (4) reduced the expression of the hypertrophic gene program with a reduction of Rcan1 expression suggesting an effect of losartan on calcineurin-dependent signaling; (4) no effects of losartan, however, were observed on genes associated with fibrosis, except for TGFβ1, nor on genes associated with inflammation or endothelial function.

Ventricular pressure overload and atrial remodeling

We observed the occurrence of several types of atrial remodeling including hypertrophy, dedifferentiation, fibrosis and inflammation. Pathological atrial hypertrophy was suggested by an increased atrial weight and increased atrial expression of BNP and skeletal α-actin. The increased expression of skeletal α-actin also suggests dedifferentiation, an important feature of remodeling in AF.36 Atrial fibrosis was suggested by increased expression of a profibrotic growth factor, the major collagens and genes involved in extracellular matrix dynamics. Inflammation was suggested by the increased expression of IL-6. Atrial hypertrophy, fibrosis and inflammation have been described in models of pressure overload before.10,11,14 Liao et al. showed an increased atrial weight, fibrosis and inflammation after 10 days of TAC.14 Increased atrial weight and fibrosis was also seen in a model with a more gradual increase in left ventricular afterload, caused by a constriction of the ascending aorta in weanling rats.11 Furthermore, sheep with hypertension caused by the one-kidney, one-clip model, showed enlarged atria, atrial fibrosis and an increased amount of inflammatory cells in the atria.10 Interestingly, atrial dilatation, atrial hypertrophy and atrial inflammation were already present after 5 weeks whereas fibrosis developed later.10 In human, data on atrial structural remodeling obtained from biopsies is more scarce, but enlarged atria, hypertrophy and fibrosis are commonly found.16,37-40
Only recently endothelial dysfunction has been linked to AF.\textsuperscript{34,35} We, however, observed no changes in markers of endothelial dysfunction, except for an increased expression of iNOS in animals subjected to TAC and treated with losartan. In spontaneous hypertensive rats increased atrial NOS activity, as well as increased atrial protein expression of iNOS and eNOS was found.\textsuperscript{41} This suggests that hypertension may induce changes in genes associated with endothelial dysfunction. Thus, in this model ventricular pressure overload is sufficient to promote atrial remodeling, however this does not include changes related to endothelial dysfunction. Possibly we did not observe changes related to endothelial dysfunction due to the relatively short time period of pressure overload.

Effects of losartan in atrial remodeling caused by ventricular pressure overload

Continuous treatment with losartan from the moment of surgery until sacrificing in this model of continuous pressure overload did not affect LV weight nor ventricular hypertrophy. These results agree with the findings of Baba et al.\textsuperscript{42}, but are in contrast to earlier findings.\textsuperscript{43} Rockman et al. subjected mice to TAC and losartan for 7 days, losartan significantly reduced heart weight in this study.\textsuperscript{43} This discrepancy may be explained by differences in the models or in losartan dose. In both studies the increase of maximum pressure was higher than in our study (60-75% increased compared to control vs. 40% in our study), and the duration was shorter (1 and 2 weeks vs. 5 weeks). In addition, Rockman et al. used a 3.5-times higher dose of losartan than in our study. Our dose was comparable to approximately 150 mg per day in humans,\textsuperscript{44} being slightly higher than the recommended dose of 100 mg per day. Our dosage was effective, as is shown in the study by Meems et al. from which a random subset of atria was used for the present study.\textsuperscript{29}

Treatment with losartan attenuated the effects of pressure overload on expression of skeletal α-actin and Rcan1. Calcineurin-dependent signaling could be a mechanisms via which losartan exerts its effects. No effects, however, could be demonstrated on gene expression related to fibrosis, inflammation or endothelial function. Our data suggest involvement of the angiotensin II receptor, but also suggest involvement of other signaling routes. ARBs have been shown effective in attenuating remodeling in overload models. In a model of spontaneous hypertension, olmesartan reduced atrial heart weight, cellular hypertrophy and fibrosis.\textsuperscript{13} Furthermore, CS-866, an ARB, reduced expression of collagen type I and III, as well as ANP expression upon aortocaval shunt in rats.\textsuperscript{45} These results are in contrast with our study. A reason for these discrepancies may be the different models used, i.e. continuous ventricular pressure overload in our study versus volume overload and spontaneous hypertensive rats, respectively. ARBs have been shown effective in reducing atrial TGFβ1 expression as well as fibrosis in a model of ventricular tachypacing.\textsuperscript{8} We also observed a reduced TGFβ1 expression with losartan, however, this was not accompanied by a significant effect on other fibrotic genes. Figure 2 shows that the variation in the TAC and losartan is less compared to the TAC group only. Although we did not observe statistical significant differences, possibly due to the small group size, losartan might affect fibrosis related gene expression.
Losartan partially reduces atrial remodeling

Results with ARBs to prevent AF, atrial remodeling and cardiovascular events in patients have been inconsistent. In patients with hypertension and left ventricular hypertrophy new-onset AF occurred less often in patients treated with losartan compared to patients that were treated with atenolol. In the GISSI-AF trial, on the other hand, which included patients with previous AF, no beneficial effects of valsartan on recurrent AF nor on atrial remodeling could be demonstrated. In addition, the ANTIPAF trial showed no beneficial effect of an ARB on prevention of paroxysmal AF. The inconsistent results might be related to a too long duration of underlying diseases and AF, which implies that the patients that were studied in those trials already had severe more or less irreversible remodeling. Furthermore, the continuous presence of underlying heart disease, i.e. ventricular pressure overload, may reduce the potential beneficial effects of upstream therapies, such as losartan in this study. Accordingly, in patients with hypertension, reduction of blood pressure may be often insufficient, causing continuation of the remodeling process. This may be one of the reasons why the clinical efficacy of upstream therapies for prevention of AF has been so disappointing. Possibly, upstream therapy with several drugs influencing different types of remodeling processes may result in a more favorable clinical outcome.

Strengths and limitations

An important limitation is that data is limited to mRNA expression levels. We have no histological data or data on protein levels. Although a fibrosis staining would strengthen the data, we observed in a previous study an increase in gene expression of genes related to fibrosis, in the absence of histologically detectable fibrosis. We therefore think that the changes related to fibrosis in the present study will also be mainly present on the mRNA level. mRNA levels do not provide information on protein levels or activity. However, mRNA levels of natriuretic peptides and skeletal α-actin have been used as markers of hypertrophy before, and the changes in mRNA levels of the fibrotic genes have been used as markers of fibrosis. The same holds for inflammation and endothelial function. Although, we think that the induced atrial changes in this model increase the AF susceptibility we did not test this in this study. It is also possible that remodeling has not progressed enough to increase the AF susceptibility. In line with this, we did not perform electrophysiological measurements. Furthermore, the study is limited by the number of animals per group; therefore smaller differences might have not reached statistical significance. Another limitation is that the constriction develops at once, in contrast to the situation in patients in which diseases usually develop gradually. Nevertheless, we think that this study provides us with valuable insights in remodeling upon continuous pressure overload and the effects of upstream therapy thereon and provides a rationale for investigating different mechanisms more thoroughly.
Chapter 4

**Conclusion**

Permanent ventricular pressure overload by TAC induced atrial remodeling, including hypertrophy, fibrosis and inflammation in the presence of continuous ventricular pressure overload. Losartan was able to reduce the expression of the hypertrophic gene program.

**Funding**

This work was supported by Abbott (Grant Number A08-424) and the Interuniversity Cardiology Institute Netherlands (ICIN). This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project COHFAR (grant 01C-203), and supported by the Dutch Heart Foundation.

**Conflict of interest**

None declared.
Losartan partially reduces atrial remodeling

References


