Left Ventricular Reverse Remodeling After Surgical Therapy for Aortic Stenosis: Correlation to Renin-Angiotensin System Gene Expression

Thomas Walther, MD, PhD; Andreas Schubert, PhD; Volkmar Falk, MD, PhD; Christian Binner, MD; Claudia Walther, MD; Nico Doll, MD; Alex Fabricius, MD; Stefan Dhein, MD, PhD; Jan Gummert, MD, PhD; Friedrich W. Mohr, MD, PhD

Background—Surgical therapy for aortic stenosis leads to reverse remodeling, with normalization of left ventricular hypertrophy (LVH). The aim of this study was to examine Renin-Angiotensin system (RAS) gene expression in this setting.

Methods—Growing sheep (n=44) underwent supracoronary aortic banding for controlled induction of LVH at the age of 6 to 8 months (A=baseline). Surgical revision to completely release the pressure gradient was performed 8.3±1 months later (B). The animals were sacrificed after another 10.1±2 months (C). Along with hemodynamic measurements, subtractive hybridization and competitive polymerase chain reaction were applied to quantify mRNA expression for angiotensin-converting enzyme (ACE) and angiotensin receptors 1 and 2 (AT1-R and AT2-R).

Results—Left ventricular mass index was 82±21 g (A), 150±33 g (B), and 78±18 g (C), P<0.01. Left ventricular function and cardiac index remained stable. Myocardial fiber diameter was 11.3±0.8 (A), 15.9±1.2 (B), and 11.4±1 (C) μm, P<0.01. Gene expression was as follows: ACE 0.8±0.05 (A), 1.3±0.08 (B), and 0.9±0.06 (C), P<0.01; AT1-R 0.7±0.06 (A), 0.9±0.07 (B), and 0.3±0.04 (C), P<0.01; AT2-R 0.5±0.05 (A), 0.2±0.04 (B), and 0.5±0.05 (C), P<0.01.

Conclusion—LVH in aortic stenosis coincides with significant alterations of the RAS. Surgical therapy leads to reverse remodeling, which is paralleled by regression of RAS gene expression. ( Circulation. 2002;106[suppl I]:I-23-I-26.)

Key Words: aortic stenosis ■ left ventricular hypertrophy ■ renin-angiotensin-system ■ gene expression

Valvular aortic stenosis (AS) is associated with pathological ventricular remodeling, leading to significant left ventricular hypertrophy (LVH). After aortic valve replacement, reverse remodeling with normalization of left ventricular mass can be anticipated, as has been documented by echocardiographic studies.1–3 However, the underlying cellular and molecular changes in the myocardium are not yet completely understood.

Among other factors, the renin-angiotensin system (RAS) has an important role in the regulation of myocardial remodeling.4,5 Its cardiac effects are transmitted via angiotensin II and angiotensin receptor subtype 1 (AT1-R), whereas for angiotensin receptor subtype 2 (AT2-R), an inhibitory role has been described in vitro.6,7 Angiotensin II can mediate fibroblast proliferation, promote fibrosis, and can directly affect the extracellular matrix (ECM).8,9 The ECM gene expression is increased in experimental LVH, and it has been shown to decrease after corrective surgical therapy.10

To date, RAS gene expression has not been analyzed in parallel with reverse remodeling of the left ventricle after corrective surgical therapy for aortic stenosis.

Methods

A standard experimental model of supracoronary banding was applied to 44 growing female Merino sheep. Approval was obtained from the governmental offices and all animals received human care in compliance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication 85 to 23, revised 1985). All surgical interventions were performed under general anesthesia using scopolamine (0.03 mg/kg), xylazin (0.22 mg/kg), and ketamine (11 mg/kg) intramuscularly for induction as well as endotracheal intubation, isoflurane at 0.5 to 2 vol.%, fentanyl (0.1 mg), and pancuronium (1 mg).

At baseline (A, ages 6 to 8 months) LVH was induced in the sheep by supracoronary banding of the ascending aorta via a left lateral thoracotomy. Corrective surgical therapy was performed 8.3±1 months later by resecting the band and thus restoring normal ascending aortic blood flow via a median sternotomy (B). The band was peeled off the aorta on the beating heart, yielding instantaneous restoration of an unobstructed lumen of the relatively short ascending aorta. Final examinations were performed after complete regression of LVH and normalization of left ventricular mass 10.1±2 months later (C).

At all interventions, hemodynamic and standard echocardiographic examinations using transthoracic and transesophageal echocardiography were performed. Sections of the left ventricular free wall were snap-frozen in liquid nitrogen and stored at −80°C. Each
animal served as its own individual control. Quantitative histological analyses were performed after hematoxylin-eosin staining using an Axioplan2 (Carl Zeiss Vision GmbH, Jena, Germany) microscope at 400x(multi) enlargement and the KS 300 Imaging System 3.0 (Carl Zeiss Vision GmbH, Eching, Germany).

Subtractive hybridization and alignment with a reference data bank (National Center for Biotechnology Information) were used to specifically delineate the sequences for angiotensin-converting enzyme (ACE) as well as for AT₁-R and AT₂-R. At invariant positions, sense and antisense primers were determined and applied. The amplified sequences were cloned using TOPO-TA cloning vector (Invitrogen, Groningen, Netherlands) and sequenced with an ABI 377 sequencer.

Frozen left ventricular tissue samples were prepared with an ultra turrax using Trizol Reagent (Life Technologies, Karlsruhe, Germany). An aliquot of total RNA (500 ng) was reversely transcribed into cDNA using reverse transcriptase (Superscript II plus, Life Technologies) and random primers (Life Technologies). To quantify mRNA expression levels, an aliquot of the cDNA was used in the real-time polymerase chain reaction (PCR) reaction containing the gene-specific primers and LightCycler®FastStart DNA Master SYBR Green I reaction mix (Roche Diagnostics, Mannheim, Germany). The specific expression levels were analyzed using LightCycler software and expressed in relation to the individual 18S rRNA intensity in relative units. Every PCR was performed 3 times, resulting in a variability of less than 10%. PCR conditions were as follows: annealing temperature 57 to 65°C and elongation time 30 to 40 seconds at 21 to 40 PCR cycles.

Results are given as mean±standard deviation. The SPSS software (SPSS Inc., Chicago, IL) was used. All results were tested for normal distribution. For comparison of mean values for the different time points, one-way ANOVA was performed. For post-hoc analysis the Bonferroni test was applied. Probability values of 0.05 or lower on two-tailed testing were considered to indicate statistical significance.

Results

Surgical Outcome

From Banding (A) until surgical correction (B), 8 of the 44 sheep (18.2%) died as a result of perforation of the ascending aorta (3), heart failure (3), a pericardial cyst (1), and respiratory infection (1). Another 9 sheep died perioperatively at surgery (3), heart failure (3), a pericardial cyst (1), and respiratory infection (1). Thus, results are given for the surviving 27 sheep that underwent all measurements until final analysis (C).

Morphology and Hemodynamics

The animals were on standard feeding, body weight increased from 31.9±5.5 (A) to 54.1±6 (B) and was 59.3±7 kg at C. Body surface area was 1.1±0.1 (A) versus 1.4±0.1 (B) and 1.5±0.2 m² (C). Left ventricular ejection fraction was 66±6 (A) versus 69±10 (B) and 62±8 (C), p=NS. Cardiac index was 3.5±1.5 (A) versus 4.2±1.3 (B) and 4.2±1.4 L/min/m² (C), p=NS, respectively. Throughout the study, circumferential wall stress was stable at 55±19 (A), 47±35 (B), and 57±18 dyn/cm² (C). Banding (A) resulted in an initial pressure gradient of 25.8±6.3 mm Hg that steadily increased parallel with growth as diagnosed on serial follow-up echocardiography. Left ventricular mass index, maximum pressure gradients and myocardial fiber diameters are given for baseline (A) left ventricular hypertrophy (B) and after reversal of LVH (C) in Figure 1.

Relation Between Ventricular Morphology and Gene Expression

The association between left ventricular mass index (LVMI) and RAS gene expression is shown in Figure 2. Between A and B, there was a parallel increase of LVMI as well as ACE and AT1-R gene expression, and between B and C there was a parallel decrease of these parameters. For AT2-R, an inverse relationship was found. As indicated in the figure, there was a strong correlation between changes in LVMI and RAS gene expression.

Discussion

Myocardial remodeling as well as reverse remodeling after therapeutic intervention for aortic stenosis have multifactorial causes, including changes in pressure load conditions and angiotensin-converting enzyme (ACE) as well as AT₁-R and AT₂-R abundance at baseline (A), after development of left ventricular hypertrophy (B), and after corrective surgical therapy (C).

<table>
<thead>
<tr>
<th></th>
<th>ACE</th>
<th>AT₁-R</th>
<th>AT₂-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (baseline)</td>
<td>0.80±0.05</td>
<td>0.65±0.06</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>B (LVH)</td>
<td>1.27±0.08*</td>
<td>0.86±0.07*</td>
<td>0.22±0.04*</td>
</tr>
<tr>
<td>C (regression)</td>
<td>0.87±0.06*</td>
<td>0.29±0.04*</td>
<td>0.53±0.05*</td>
</tr>
</tbody>
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* p<0.01 versus previous time point.

ACE = angiotensin-converting enzyme; AT₁-R = angiotensin receptor subtype 1; AT₂-R = angiotensin receptor subtype 2; LVH = left ventricular hypertrophy.
interplay of different growth effectors and inhibitors. Myocardial remodeling is a major determinant for patient outcome because it is associated with increased morbidity and mortality.11–13

There were 3 important findings of this study: a significant increase (ACE, AT1-R) or decrease (AT2-R) of gene expression in parallel with controlled induction of compensated LVH; complete regression of RAS gene expression after corrective surgical therapy; and the correlation between changes in left ventricular mass index and RAS gene expression.

The impact of the RAS on myocardial remodeling has been outlined in several studies.4,8,14–19 Myocardial remodeling is associated with an increase in ACE and AT1-R, as well as a low level of AT2-R gene expression. This has been explained by the specific antiproliferative effects of the AT2-R and the potential for antagonizing AT1-R-mediated effects.16,19 We observed a similar inverse regulation of AT1-R and AT2-R gene expressions as well as reversal after corrective surgical therapy. In contrast to these findings, an obligatory requirement for AT2-R has been suggested recently for the development of LVH.20 However, results from different studies applying different models are divergent, and the specific impact of the AT2-R on myocardial remodeling remains to be proven.21

The chosen model allowed to mimic the clinical situation of aortic stenosis. The atypical coronary perfusion pattern was taken into account for us to have a rather simple model ensuring the survival of the animals. The use of growing sheep was important to achieve a gradual increase in pressure gradient after banding. Thus, compensated LVH was obtained. The major limitation of the sheep model was the lack of suitable antibodies to determine protein levels.

By intention, the present study was performed without additional medical therapy to document the single effect of surgical intervention. Additional medical therapy with an ACE inhibitor or an AT1-R antagonist could be an efficient strategy to enhance regression of LVH after surgical therapy for aortic stenosis.

The balanced increase in RAS gene expression is in accordance with the hemodynamic findings of compensated LVH. Within the time frame chosen, no significant fibrosis occurred in our model. This is in contrast to more advanced stages of LVH, where the presence of fibrosis is thought to be the reason for incomplete regression.1 Complete LVH regression may therefore only occur in the absence of significant fibrosis. Myocardial reverse remodeling may be affected by multiple growth effectors and inhibitors, as well as age, sex, other cardiovascular risk factors and the model chosen.

In summary, in a model of compensated LVH it has been shown that myocardial remodeling as well as RAS gene expression are reversible after corrective surgical therapy. The change in RAS gene expression can be considered part of the adaptive process of the left ventricle. After corrective surgical therapy for aortic stenosis, medical therapy modulating the RAS may be an additional attractive strategy to enhance ventricular reverse remodeling.

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References
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