Effect of the Angiotensin-converting Enzyme Inhibitor Alacepril on Ventricular Function and Beta-Adrenoceptor Number in Rabbits with Aortic Regurgitation

Tsutomu YOSHIKAWA, M.D., Shunnosuke HANIDA, M.D.,
Keiichi NAGAMI, M.D., Masahiro SUZUKI, M.D.,
Yumiko WAINAI, M.D., Takeo MINAMI, Ph.D.,*
Kazutoshi SUZUKI, Ph.D.,*
and Shikifumi KITAZAWA, Ph.D.*

SUMMARY

This study was performed to determine the effects of the angiotensin-converting enzyme inhibitor alacepril on hemodynamic variables and beta-adrenoceptor number in rabbits with heart failure induced by aortic regurgitation. Aortic regurgitation was induced by perforation of the aortic valve in 12 rabbits. Sixty mg/kg of alacepril was administered by gastric tube for 7 days after manifestation of aortic regurgitation to 6 rabbits (group AR + A). The other 6 rabbits with aortic regurgitation were administered vehicle in the same fashion (group AR + C). Seven rabbits underwent sham operation (group S). One week after induction of aortic regurgitation left ventricular end-diastolic pressure was higher and cardiac output was lower in AR + C than in S. End-diastolic and end-systolic left ventricular diameter were larger and left ventricular weight was also higher in AR + C than in S. For each of these parameters, the opposite findings were obtained from a comparison of AR + A and S. Myocardial beta-adrenoceptor density and norepinephrine content were reduced in AR + C, but were restored in AR + A. These findings indicate that alacepril has beneficial effects on ventricular remodeling and function, and on sympatho-neuronal regulation in the volume-overloaded myocardium. (Jpn Heart J 36: 91-100, 1995)

Key words: aortic regurgitation heart failure angiotensin-converting enzyme inhibitor beta-adrenoceptor catecholamine

The renin-angiotensin system is activated in patients with heart failure, and protects against poor organ perfusion in the presence of low cardiac output. Its activation can also result in excess vasoconstriction, which exacerbates ad-
Advanced heart failure. Angiotensin-converting enzyme inhibitors have been reported to have beneficial effects in patients with heart failure, including improvement of exercise capacity\textsuperscript{1,2} and prolongation of survival\textsuperscript{3,4}. Their effects are clinically superior to those of other vasodilating agents even when the hemodynamic status is comparable\textsuperscript{5,6}. In addition, the findings of the CONSENSUS study have shown that they are particularly useful for patients with severe congestive heart failure in whom neurohumoral activation has occurred\textsuperscript{7}. Angiotensin-converting enzyme inhibitors interact with the sympathetic nervous system to produce a variety of effects, including inhibition of norepinephrine release from the nerve terminal\textsuperscript{8} and baroreceptor sensitization\textsuperscript{9}, as well as direct elimination of excessive vasoconstriction.

We previously reported that myocardial beta-adrenoceptors were down-regulated and myocardial catecholamines were depleted in rabbits with left ventricular failure following the induction of aortic regurgitation (AR)\textsuperscript{10}. The angiotensin-converting enzyme inhibitor alacepril is metabolized \textit{in vivo} to captopril and desacetylalacepril. It has an inhibitory effect on sympathetic vasoconstriction together with inhibition of the angiotensin-converting enzyme\textsuperscript{11}. The present study was designed to determine the effect of alacepril on hemodynamic variables and myocardial beta-adrenoceptors and catecholamines in volume-overloaded myocardium in rabbits.

\textbf{Methods and Materials}

\textbf{Induction of AR:} AR was induced as previously described\textsuperscript{12,13}. Twelve female Japanese white rabbits were anesthetized with a 0.22 mg/kg intravenous injection of chloral hydrate. The right carotid artery was isolated from the surrounding tissue. Aortic root pressure was measured with a 5F micromanometer-tipped catheter introduced through the carotid artery. The metal catheter was introduced from the right carotid artery, and advanced to the aortic root, where the motion of the aortic valve could be detected through the catheter. The metal catheter was pushed toward the left ventricle, and the aortic valve was perforated. Successful induction of AR was confirmed by the appearance of a diastolic murmur. Aortic pressure was measured again to assess the severity of AR\textsuperscript{14}. The right carotid artery was ligated, and the surgical incision closed. Antibiotics were injected post-surgically. Sham operation was performed in 7 other rabbits, in which the right carotid artery was ligated, but AR was not induced.

\textbf{Drug administration:} Alacepril was dissolved in 0.5% tragacanth solution as previously described\textsuperscript{15}. Sixty mg/kg of alacepril was administered once a day via a gastric tube for 1 week to 6 rabbits with AR (group AR+A). Tragacanth solution (0.5%) was administered in the same fashion as a vehicle to the remain-
ing 6 rabbits with AR (group AR + C).

**Hemodynamic measurement:** Hemodynamic measurement was performed in the open-chest anesthetized condition 2 hours after the final administration of alacepril or vehicle solution. Rabbits were anesthetized with an intravenous injection of chloral hydrate. Ventilation was performed via tracheal incision. Tidal volume was adjusted to 50 ml with a frequency of 15/min. After the midline thoracotomy, the pericardium was opened. Aortic pressure was monitored with a 5F micromanometer-tipped catheter introduced through the left carotid artery. Aortic flow was measured with an electromagnetic flow probe placed around the aortic root. Left ventricular diameter was measured with a pair of ultrasonic crystals attached to the anterior and posterior epicardial surfaces. The left ventricular pressure and its first derivatives (dp/dt) were measured with a 3F micromanometer-tipped catheter introduced through the apical incision. After measurement of hemodynamic variables, the heart was stopped by the rapid injection of potassium chloride. Blood was withdrawn from the right ventricle for determination of plasma captopril concentration. The left ventricular myocardium was isolated for weighing and measurement of its thickness. Approximately 0.5 g of the left ventricular myocardium was rapidly frozen with liquid nitrogen and stored at -80°C until assay of myocardial catecholamines by high performance liquid chromatography. Plasma catecholamine levels were not determined in this study.

**Membrane preparation:** The left ventricular myocardium was soaked in ice-cold isotonic sucrose buffer containing 0.25 M sucrose, 1 mM KHCO₃, and 1 mM MgCl₂. Connective tissue, pericardium, and endocardium were removed as much as possible, and the remaining tissue was then minced with scissors, homogenized with a polytron (Phycotron, Niti-on) at maximum speed for 10 seconds, and centrifuged at 700 g for 10 minutes. Supernatants were collected and centrifuged further at 17,000 g for 15 minutes. Pellets were resuspended in 4 ml of ice-cold Tris buffer containing 100 mM Tris, 1 mM MgCl₂, and 5 mM EGTA. They were frozen rapidly with liquid nitrogen, and stored at −80°C until assay. Protein concentration in the membrane fraction was determined by the modified Lowry method.

**Assay of beta-adrenoceptors:** The protein concentration of the membrane sample was adjusted to 0.3 mg/ml. ¹²⁵I-iodocyanopindolol (Amersham Japan) was used for the radioligand binding assay for beta-adrenoceptors. Twenty-five µl of 30–800 pM ¹²⁵I-iodocyanopindolol with 25 µl of ice-cold Tris buffer or 10⁻⁵ M propranolol was added to 100 µl of membrane sample. This mixture was incubated at 37°C for 60 minutes. Reaction was terminated by adding 750 µl of ice-cold Tris buffer. Samples were filtered through a Whatman GF/C glass fiber filter and washed twice with 5 ml of ice-cold Tris buffer. Radioactivity was
counted with an ARC-600 gamma counter with 76.5% counting efficiency. Membrane-bound specific activity was determined by subtraction of nonspecific binding in the presence of propranolol from total binding. The number of maximal binding sites and dissociation constant were determined by Scatchard analysis.19)

Data analyses: Hemodynamic data were recorded by a thermal array recorder (Nihon-Kohden Inc.) at 200 mm/sec paper speed. Total forward stroke volume (TSV) and regurgitant volume (RV) were calculated from digitization of the positive and negative components of aortic flow using the MYPAD-A3 Logitec DIGITIZER (MODEL K-510). Stroke volume was calculated by subtraction of RV from TSV, and regurgitant fraction (RF) was calculated as follows:

$$RF(\%) = \frac{RV}{TSV} \times 100$$

The least squares method was used to analyze the Scatchard plot.

Statistical analyses: Results are expressed as mean ± SD. Analysis of variance and Student t-test were used for comparison among groups. Differences were considered statistically significant when $p < 0.05$.

Results

Plasma and myocardial captopril concentrations were 11.4 ± 5.1 µg/ml and 1.8 ± 1.0 µg/g tissue in alacepril-treated rabbits. Table I shows the results of

<table>
<thead>
<tr>
<th>Table I. Hemodynamic Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>BW (kg)</td>
</tr>
<tr>
<td>AEDP (mmHg)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
</tr>
<tr>
<td>Ao systolic (mmHg)</td>
</tr>
<tr>
<td>diastolic (mmHg)</td>
</tr>
<tr>
<td>mean (mmHg)</td>
</tr>
<tr>
<td>+dp/dt (mmHg/sec.div)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
</tr>
<tr>
<td>EDD (mm)</td>
</tr>
<tr>
<td>ESD (mm)</td>
</tr>
<tr>
<td>CO (ml/min/kg)</td>
</tr>
<tr>
<td>SV (ml/kg)</td>
</tr>
<tr>
<td>RV (ml/kg)</td>
</tr>
<tr>
<td>RF (%)</td>
</tr>
</tbody>
</table>

S = sham-operated rabbits; AR + C = rabbits with AR given vehicle; AR + A = rabbits with AR given alacepril; BW = body weight; AoDP = decrease in aortic diastolic pressure after production of AR; Ao = aortic pressure; LVEDP = left ventricular end-diastolic pressure; EDD and ESD = end-diastolic and end-systolic diameter; CO = cardiac output; SV = stroke volume; RV = regurgitant volume; RF = regurgitant fraction. * = $p < 0.05$ compared with S; ** = $p < 0.01$ compared with S; + = $p < 0.05$ compared with AR + C.
hemodynamic measurements for the three groups. Body weight was lower in both AR + C and AR + A than in the sham-operated rabbits. However, there was no difference in body weight between the two groups with AR. Aortic diastolic pressure was lower in both AR + C and AR + A than in the sham-operated rabbits, but did not differ between AR + C and AR + A. There was no difference in degree of reduction of aortic diastolic pressure following induction of AR between the two groups. AR + C and AR + A also had similar regurgitant fractions and regurgitant volumes. There was no difference in + dp/dt between AR + C and the sham-operated rabbits, but + dp/dt was lower in AR + A than in the sham-operated rabbits. The left ventricular end-diastolic pressure was higher in AR + C than in the sham-operated rabbits. There was no difference between AR + A and the sham-operated rabbits in left ventricular end-diastolic pressure. End-diastolic left ventricular diameter was larger in AR + C than in the sham-operated rabbits, but was smaller in AR + A than in AR + C. End-systolic diameter was also larger in AR + C than in the sham-operated rabbits, but did

**Figure.** Upper panel shows maximal binding sites of beta-adrenoceptor (Bmax). Bmax was lower in AR + C than in S, but was higher in AR + A than in AR + C. As shown in the lower panel, dissociation constant (Kd) was higher in AR + A than in AR + C. * = $p < 0.05$ compared with S; ** = $p < 0.01$ compared with S; ** = $p < 0.01$ compared with AR+C.
not differ between AR + A and the sham-operated rabbits. Cardiac output and stroke volume were lower in AR + C than in the sham-operated rabbits, but neither differed between AR + A and the sham-operated rabbits. Left ventricular weight was higher in AR + C than in the sham-operated rabbits (1.50 ± 0.17 vs. 1.30 ± 0.07 g/kg), but did not differ between AR + A (1.39 ± 0.20) and the sham-operated rabbits. Wall thickness was higher in AR + C than in the sham-operated rabbits, but did not differ between AR + A and the sham-operated rabbits.

The figure shows the results of myocardial beta-adrenoceptor assay. Numbers of maximal binding sites of myocardial beta-adrenoceptors were lower in AR + C (39.6 ± 5.3 fmol/mg protein) than in the sham-operated rabbits (69.9 ± 11.2), but were higher in AR + A (56.3 ± 7.7) than in AR + C. Dissociation constant was higher in AR + A than in AR + C. Table II shows findings for myocardial catecholamine content. Myocardial norepinephrine level was lower in AR + C than in the sham-operated rabbits, but did not differ between AR + A and the sham-operated rabbits.

**DISCUSSION**

We demonstrated that the angiotensin-converting enzyme inhibitor alacepril prevented left ventricular enlargement and improved hemodynamic variables following induction of AR in rabbits. It also reversed down-regulation of myocardial beta-adrenoceptors and depletion of myocardial norepinephrine.

**Effects of AR on hemodynamics and beta-adrenoceptors:** Hemodynamic findings showed that 1 week after induction of AR, left ventricular end-diastolic pressure was elevated, and cardiac output was reduced despite ventricular enlargement, suggesting impairment of left ventricular contractile performance. Down-regulation of myocardial beta-adrenoceptors and depletion of catecholamines were also observed. In a previous study we found that left ventricular end-diastolic pressure was elevated, and beta-adrenoceptor number was reduced only 1 day after induction of AR. However, neither cardiac output nor myocardial catecholamine levels were reduced at that time. Left ventricular function and sympathetic-neuronal regulation progressively deteriorated during the week following induction of AR. Gilson et al reported that myocardial beta-
adrenoceptor number was reduced in rabbits with pressure and volume overload. In contrast, Yamazaki et al.\(^{22}\) reported that numbers of myocardial beta-adrenoceptors were increased in rats with AR. Florenzano et al.\(^{23}\) reported that left ventricular contractility was increased following induction of AR in chronically instrumented conscious dogs. However, AR induced in our study was severe enough to explain this discrepancy in findings. AR can if severe enough, result in left ventricular failure in animal models.

**Mechanisms of improved hemodynamics and beta-adrenoceptors:**
Alacepril is an angiotensin-converting enzyme inhibitor, the effects of which are mediated by a metabolite, captopril. Desacetylalacepril, another metabolite of alacepril,\(^{24}\) has been found to attenuate the vasoconstrictive response to norepinephrine.\(^{25}\) Alacepril therefore exhibits both potent sympatho-inhibitory activity and inhibition of angiotensin-converting enzyme. Three explanations are possible for the improved hemodynamics and beta-adrenoceptors we observed: 1) direct vasodilation; 2) effects on sympatho-neuronal regulation; and 3) prevention of left ventricular remodeling. It is possible that alacepril dilated arteriolar resistance vessels resulting in a decrease in regurgitant volume. However, no significant difference was found between treated and nontreated rabbits in regurgitant fraction in our study. Gay\(^{26}\) reported that the pressure-volume curve shifted to the left in captopril-treated AR rats. He found no decrease in regurgitant volume, and presented no findings concerning sympatho-neuronal regulation. Clinical trials in patients with heart failure have shown that angiotensin-converting enzyme inhibitors increase survival more than other vasodilating agents.\(^{5,6}\) These agents are especially useful in the treatment of patients with severe congestive heart failure in whom neurohumoral activation has occurred.\(^{7}\) This emerging evidence suggests that other undefined mechanisms such as attenuation of sympatho-neuronal activities or prevention of ventricular remodeling are possible. Animal studies have shown that angiotensin-converting enzyme inhibitors induce up-regulation or enhanced beta-adrenergic responsiveness in intact or failing myocardium.\(^{27,28}\) A recent study\(^{29}\) demonstrated that the angiotensin-converting enzyme inhibitor lisinopril increased myocardial beta-adrenoceptor density in the failing human heart, although the hemodynamic changes induced by lisinopril were insignificant. Augmentation of sympatho-neuronal transmission occurred after induction of AR, resulting in down-regulation of beta-adrenoceptors and depletion of catecholamines.\(^{10,20}\) It is possible that alacepril directly eliminated excessive activation of sympatho-neuronal transmission after induction of AR and protected the myocardium from excessive exposure to catecholamines. On the other hand, angiotensin-converting enzyme inhibitors have been shown to prevent ventricular remodeling in experimental myocardial infarction.\(^{30,31}\) Favorable effects of these drugs have also been obtained in clinical
settings.\textsuperscript{32-35} It is possible that attenuation of ventricular remodeling following AR prevented ventricular dilatation and dysfunction, resulting in restoration of myocardial beta-adrenoceptors and depletion of myocardial catecholamines.

**Limitations of this study:** Differences in findings between the two groups with AR were not attributable to differences in severity of AR, since the decrease in aortic diastolic pressure after induction of AR, which is predictive of subsequent left ventricular overload,\textsuperscript{14} was similar in the two groups. Aortic diastolic pressure and regurgitant fraction were also similar for the two groups 1 week after induction of AR. We did not, however, determine the sequential changes which occurred in these variables during the week after induction of AR. Thus there was no difference in severity of volume overloading between the two groups. Favorable findings for the treated group appear to be caused by the effects of alacepril itself.

**ACKNOWLEDGMENT**

Alacepril was kindly provided by Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. We gratefully acknowledge their helpful advice and encouragement in performing this study.

**REFERENCES**

10. Yoshikawa T, Handa S, Yamada T, Wainai Y, Suzuki M, Tani M, Nakamura Y: Role of adrenergic-

