Effects of Losartan, an Angiotensin II Antagonist, on the Development of Cardiac Hypertrophy Due to Volume Overload

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To investigate the contribution of a cardiac renin-angiotensin system to cardiac hypertrophy due to volume overload, the effects of losartan, a non-peptide angiotensin (Ang) II type 1 (AT₁) receptor antagonist, on left ventricular hypertrophy (LVH) was studied. LVH was produced in male Wistar rats by volume overload secondary to aortic insufficiency (AI). Losartan (10 mg/kg/d) was orally administered for 2 weeks after surgery to both AI and sham-operated (control) rats. Two weeks after surgery, aortic pulse pressure and left ventricular (LV) weight were markedly increased in the AI rats as compared with the control group, whereas cardiac angiotensin converting enzyme (ACE) activity remained unchanged. The effects of the chronic administration of losartan on AT₁ receptors were verified by the blockade of Ang II pressor response. Losartan treatment produced a significant reduction in LVH in AI rats without affecting the systolic blood pressure. In separate groups of rats, to elucidate the mechanisms of the attenuation of LVH by treatment with losartan, we determined plasma and LV immunoreactive Ang II content and plasma renin activity (PRA). LV Ang II content increased in AI rats, while plasma Ang II content, PRA and serum ACE activity did not. Losartan significantly reduced the LV Ang II content, whereas PRA and plasma Ang II concentration were increased by the treatment. There was a significant positive correlation between LV weight and LV Ang II content. These results suggest that cardiac Ang II, rather than circulating Ang II, plays an important role in the LVH due to volume overload via the AT₁ receptor.

Key words cardiac hypertrophy; volume overload; angiotensin II; losartan

In response to mechanical overloading, such as pressure or volume overload, left ventricular hypertrophy (LVH) is induced to adapt to increased loads through different processes, and LVH has been recognized as a serious risk factor for severe cardiac dysfunctions. The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure, electrolyte and fluid homeostasis. Local tissue RAS have recently been discovered in various organs, including the heart. Angiotensin (Ang) II is a key effector molecule of the RAS. There are two types of Ang II receptors, AT₁ and AT₂, through which Ang II produces its biological effects. It has been shown that almost all the systemic effects of Ang II are mediated through AT₁ receptors.

Vascular Ang II receptors are thought to be predominantly AT₁ in adult rats. Recently, Dostal et al. have shown that components of RAS, renin and angiotensinogen, are present in neonatal rat heart, and they detected Ang I, Ang II and angiotensin converting enzyme (ACE) activity in cultured cardiac myocytes and fibroblasts. It has also been shown that Ang II directly stimulates protein synthesis and cell growth in cultured myocytes, and induces cardiac hypertrophy in vivo via AT₁ receptors. There is increasing evidence that locally produced Ang II contributes to cardiac hypertrophy in spontaneously hypertensive rats (SHR) or in aortic-constricted rats. These findings suggest that cardiac RAS may be involved in the progress of pressure overload cardiac hypertrophy, presumably via AT₁ receptors. On the other hand, the involvement of cardiac RAS in volume overload cardiac hypertrophy is not clear at present. Gay and Umemura et al. have demonstrated that the development of LVH due to volume overload in rats with aortic insufficiency (AI) was suppressed by ACE inhibitor treatment. However, they did not investigate any possible changes in the activity of systemic or local RAS.

In this study we have investigated whether RAS, via AT₁ receptors, is involved in the development of volume overload cardiac hypertrophy by using a non-peptide Ang II type 1 (AT₁) receptor specific antagonist, losartan. Cardiac hypertrophy was induced in Wistar rats by volume overload secondary to aortic insufficiency. Ang II content, ACE activity and plasma renin activity (PRA) were also measured.

MATERIALS AND METHODS

Animal Preparation AI was produced in male Wistar rats (270—290 g), using previously described techniques with some modifications. Each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) prior to operation. A fluid-filled polyethylene catheter connected to a pressure transducer was inserted into the right common carotid artery and advanced to the aortic valve to produce AI by perforating the cusps, while simultaneously monitoring aortic pressure. The establishment of AI was confirmed by an increase in the aortic pulse pressure by more than 50% of the pre-operation value. Rats treated in the same way without any perforation of the cusp served as sham-operated controls. After operation, the rats were housed in a temperature, humidity and light-controlled room for two weeks with regular rat chow and water ad libitum. All experimental procedures were approved by the Ethical Committee of Hamamatsu University School of Medicine.

The following four groups of rats were studied: group 1, control (n = 5), group 2, control receiving 10 mg/kg/d losartan for 2 weeks (n = 5), group 3, AI rats (n = 5),
group 4, Al rats receiving 10 mg/kg/d losartan for 2 weeks (n=6). Losartan was dissolved in distilled water and administered orally every day (1 ml/kg). Administration of losartan was started 1 d after the operation. The dose of losartan was decided from the results of our previous experiment. Treatment with losartan for 2 weeks, at this dose, neither lowered blood pressure nor reduced cardiac mass in normal rats (unpublished data). Further, Wong et al.20 have reported that DuP 753 (losartan) did not lower blood pressure in conscious normotensive rats, even at a dose of 10 mg/kg i.v.

Assessment of the Effect of Losartan on Ang II Pressor Response After the last administration of losartan, the rats were anesthetized and fluid-filled catheters were inserted into the femoral artery and vein. The catheters were tunneled subcutaneously and exteriorized at the neck. After the rats had recovered overnight, the arterial catheter was connected to a pressure transducer to measure the basal blood pressure. Ang II (0.1 μg/kg) was administered via the venous catheter into conscious rats, and the change in mean aortic blood pressure was monitored. After assessment of the effect of losartan on pressor response to Ang II 24 h after administration, each rat was anesthetized and the heart was excised and divided into the atria, right and left ventricles and was weighed after being washed with cold saline, then frozen in liquid nitrogen and stored at -70°C for subsequent determination of tissue ACE activity.

Measurement of ACE Activity Cardiac and serum ACE activity was measured according to the fluorometric method described by Cheung and Cushman.21 Frozen tissue was homogenized in 10 volumes of ice cold buffer (100 mm potassium phosphate buffer (pH 8.3) containing 300 mm NaCl) using a Polytron homogenizer (PT-10/35; Kinematica, Switzerland), and the homogenate was centrifuged at 4°C for 20 min at 10000 x g. The ACE activity of the supernatant was determined by using 5 μM Hip-His-Leu as a substrate. One unit of ACE activity was defined as the amount (in μmol) of His-Leu liberated per min at 37°C. Tissue ACE activity is expressed as milli units (mU) per mg protein. The protein concentration of the supernatant was determined according to the method of Bradford,22 using Bio-Rad’s Protein Assay Kit, and gamma globulin was used as a standard protein.

Analysis of Cardiac and Plasma Ang II Content and PRA Cardiac and plasma immunoreactive Ang II content and PRA were determined in three groups of animals: controls, AI and losartan treated AI. Two weeks after the same surgical procedure described above, the animals were anesthetized, blood samples were taken from the abdominal aorta, and left ventricular (LV) tissues were weighed and frozen as described above. The extraction of tissue Ang II was performed essentially according to the method of Phillips and Stenstrom.23 Frozen tissues were homogenized in 10 volumes of 1 N AcOH containing 10 μg/ml of pepstatin and concentrated by passing through C-18 Sep-Pak cartridges (Waters, U.S.A.). Ang II content was determined by radioimmunoassay (RIA) using an RIA kit (Nichols Institute, Netherlands) according to the manufacturer’s recommendations. Blood samples were collected into tubes containing aprotinin (300 kallikrein inactivating units/ml) and Na2EDTA (1 mg/ml), and plasma was subjected to RIA as described above. PRA was determined by RIA of Ang I using a commercially available kit, RENIN-RIA BEAD® (Dainabot Co., Ltd., Tokyo).

Materials Ang II, Hip-His-Leu, and His-Leu were purchased from Peptide Institute (Osaka, Japan). Aprotinin and pepstatin were purchased from Boehringer Mannheim GmbH. Losartan (or DuP753) (2-n-butyl-4-chloro-5-hydroxymethyl-1-{[(2-(1H-tetrazol-5-yl)bi phenyl-4-yl)methyl]-imidazole potassium) was provided by Banyu Pharmaceutical Co.

Statistical Analysis All values are expressed as the mean ± S.E.M. Statistical analysis was performed using ANOVA followed by Scheffe’s multiple range test. A p value <0.05 was considered significant.

RESULTS

Effects of Losartan on the Pressor Response to Ang II Responses to exogenously administered Ang II (0.1 μg/kg i.v.) measured in conscious rats 24 h after the last administration of losartan are shown in Fig. 1. The pressor response to Ang II was reduced in Al rats compared with the control rats (p<0.01), and it was further reduced by treatment with losartan (p<0.05).

Effects of Losartan on LV Weight, Blood Pressure and ACE Activity The effects of losartan on LV weight, blood pressure and LV ACE activity are summarized in Table 1. LV weight (mg/g body weight, LVW/BW) increased significantly in Al rats as compared with control group. Aortic systolic blood pressure significantly decreased in Al rats, while heart rate did not change significantly within each group. Aortic pulse pressure was significantly increased in Al rats compared with the control rats. By treatment with losartan, LVW/BW was reduced significantly in Al rats, whereas systolic blood pressure did not change. LV ACE activity did not change significantly in any group.

Effects of Losartan on Ang II Content and PRA Using another 3 groups of rats (control, Al, and losartan treated

![Graph](image-url)

Fig. 1. Effect of Volume Overload and Losartan Treatment on the Pressor Response to Ang II (0.1 μg/kg i.v.) in Conscious Control and Al Rats. Treated with (+L) or without Losartan

MAP means mean arterial pressure. Values represent the mean ± S.E.M., n=5-6 per group. ** p<0.01 vs. control, # p<0.05 vs. AI.
Table 1. Body Weight, Hemodynamic Values and LV ACE Activity in Rats Treated with or without Losartan

<table>
<thead>
<tr>
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<th>Control</th>
<th>AI</th>
<th>Control + L</th>
<th>AI + L</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>284.5 ± 2.9</td>
<td>288.8 ± 4.2</td>
<td>290.6 ± 1.9</td>
<td>282.1 ± 1.9</td>
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<tr>
<td>LVW/BW (mg/g)</td>
<td>1.77 ± 0.04</td>
<td>2.52 ± 0.10</td>
<td>1.71 ± 0.04</td>
<td>2.20 ± 0.16</td>
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<tr>
<td>HR (beats/min)</td>
<td>365 ± 16</td>
<td>339 ± 10</td>
<td>366 ± 18</td>
<td>354 ± 22</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134.4 ± 4.5</td>
<td>117.7 ± 0.7</td>
<td>127.4 ± 5.7</td>
<td>120.6 ± 3.1</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>40.9 ± 1.3</td>
<td>59.5 ± 2.0</td>
<td>38.9 ± 0.9</td>
<td>60.1 ± 5.3</td>
</tr>
<tr>
<td>LV ACE activity</td>
<td>0.048</td>
<td>0.049</td>
<td>0.051</td>
<td>0.052</td>
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<tr>
<td>(mU/mg protein)</td>
<td>±0.003</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.005</td>
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<td>n</td>
<td>5</td>
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Values are means ± S.E.M. Control, sham-operated rats; AI, aortic insufficient rats; HR, heart rate; L, losartan; LVW/BW, left ventricular weight/body weight. 
a) p<0.05 as compared with control rats. b) p<0.01 as compared with control rats. c) p<0.05 as compared with untreated AI rats.

Table 2. LV and Plasma Immunoreactive Ang II Levels, Plasma Renin Activity and Serum ACE Activity in Control, AI, and Losartan Treated Al Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AI</th>
<th>Al+losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVW/BW (mg/g)</td>
<td>1.76 ± 0.02</td>
<td>2.67 ± 0.11</td>
<td>2.36 ± 0.08</td>
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<tr>
<td>LV IR-Ang II (pg/tissue)</td>
<td>39.8 ± 5.8</td>
<td>60.8 ± 5.8</td>
<td>39.9 ± 3.4</td>
</tr>
<tr>
<td>Plasma Ang II (pg/ml)</td>
<td>57.5 ± 17.9</td>
<td>64.8 ± 20.3</td>
<td>107.5 ± 25.9</td>
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<tr>
<td>PRA (ng/ml/h)</td>
<td>3.2 ± 0.5</td>
<td>3.7 ± 0.5</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td>Serum ACE (mU/ml)</td>
<td>70.1 ± 5.1</td>
<td>73.0 ± 3.6</td>
<td>73.6 ± 7.2</td>
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<tr>
<td>n</td>
<td>5</td>
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Values are means ± S.E.M. Control, sham-operated rats; AI, aortic insufficient rats; IR-Ang II, immunoreactive Ang II; PRA, plasma renin activity, expressed as ng Ang I released from 1 ml plasma per h. a) p<0.05 as compared with control rats. b) p<0.01 as compared with control rats. c) p<0.05 as compared with untreated AI rats.

Al groups), we determined the LV and plasma immunoreactive Ang II content, PRA and ACE activity to estimate the relative activities of cardiac and circulating RAS. The results obtained from these groups are presented in Table 2. In AI rats, the LV immunoreactive Ang II content increased significantly as compared with the control rats, whereas the PRA and plasma immunoreactive Ang II content did not change significantly. Losartan significantly decreased LVW/BW, which was associated with a significant reduction in LV immunoreactive Ang II content. Losartan treatment increased PRA from 3.7 ± 0.5 to 6.6 ± 1.2 (ng Ang I/ml/h) (p<0.05). There was no significant difference in serum ACE activity among the groups. As shown in Fig. 2, there was a significant positive correlation between LVW/BW and LV Ang II content with respect to the control and AI rats. In contrast, the values of the losartan treated AI rats were not as closely correlated; Ang II content decreased to the same level as the control rats, whereas LVW/BW decreased moderately.

**DISCUSSION**

In the present study, we investigated the effects of a non-peptide Ang II receptor antagonist, losartan, which is specific for AT1 type receptors, on the development of volume overload cardiac hypertrophy in AI rats. With this treatment, we attempted to evaluate the role of Ang II in cardiac hypertrophy via AT1 receptors. The AT1 receptor antagonist losartan has been reported to inhibit Ang II induced hypertrophy of cardiac myocytes241 and of LVH in rat heart.123 In line with these reports, we demonstrated in the present study that treatment with losartan resulted in the attenuation of LVH in AI rats. This effect reflected the decrease in LV immunoreactive Ang II content. These findings suggest that Ang II may be involved in LVH due to volume overload through AT1 receptors.

In order to obtain an insight into the mechanisms underlying the effects of losartan on cardiac hypertrophy, we examined changes in the cardiac and systemic RAS and also the effects of chronic losartan treatment on these changes. The dose of losartan was chosen so that it did not lower blood pressure, but inhibited the pressor response to exogenous Ang II. The effect of losartan was verified by a decreased reactivity to administered Ang II, as shown in Fig. 1. Reduced reactivity to added Ang II was also observed in AI rats, so this might be specific to AI rats, because we observed arterial dilatation and decreased ACE activity in AI rats (unpublished observation).

The elevation of ACE activity in the ventricles of pressure overloaded or myocardial infarcted rat heart has been reported previously,15,26 but there has been no report on changes in ACE activity in a volume overloaded heart.
In contrast to the results obtained with a pressure overloaded heart, ventricular ACE activity did not increase in AI rats. Similarly, there was no significant difference in serum ACE activity and PRA between control and AI rats. PRA was increased by treatment with losartan in AI rats. The increases in PRA were considered to be due to the blockade by losartan of the negative-feedback action of Ang II on renal renin secretion.27 We found that there was a significant difference in LV Ang II content between the control and AI rats. These results suggest that there was no significant difference in RAS between the control and AI rats, except for LV Ang II content, at least 2 weeks after operation. Losartan treatment significantly attenuated the development of LVH, as well as the increase in LV Ang II content in AI rats. Similar results have also been demonstrated in losartan treated14 or ACE inhibitor treated13 SHR. The mechanism by which LV Ang II content is reduced by losartan is not clear. It may be possible that losartan suppressed the cardiac renin expression, because Ang II can be generated from Ang I by an alternative pathway in the heart.28 A recent study, which showed that Ang I, as well as Ang II, content in the LV of SHR was significantly reduced, but PRA was significantly increased by treatment with an another Ang II receptor (AT1) antagonist, TCV-116,291 suggests that cardiac, but not renal, renin activity was decreased via the blockade of AT1 receptors. This finding also indicates that cardiac Ang II contributes to LVH in an autocrine/paracrine fashion without exerting a negative feedback regulation on the expression of cardiac renin.

Although losartan treatment completely attenuated the increase in LV Ang II content, LVH was attenuated incompletely in AI rats, in spite of the fact that the LV Ang II contents were positively correlated with LV weight in the control and AI rats. Therefore, it appears that cardiac Ang II partially contributes to the development of volume overload cardiac hypertrophy and that some factors other than endogenous Ang II also may participate in this process. It is suggested that transforming growth factor-β1 or endothelin-1 are also involved in cardiac hypertrophy in combination with endogenous Ang II.30,31 Further study will elucidate the precise role of cardiac Ang II in the process of LVH.

On the other hand, Ruzicka et al.321 have evaluated the effect of losartan in volume overload cardiac hypertrophy produced by abdominal aorticaval shunt. They have shown that increases in both LV and right ventricular (RV) masses were significantly attenuated by losartan treatment; contrary to this report, RV hypertrophy was not significantly regressed in our model (data not shown). This may be attributable to the differences in drug dosage and surgical procedure between the two models. Ruzicka et al.321 used a higher dose of losartan, 40 mg/kg p.o., as opposed to the 10 mg/kg p.o. used in our study. Further, they produced volume overload by abdominal aorticaval shunt, which mainly affects the RV function, whereas, we produced volume overload due to aortic insufficiency, which mainly affects the LV function. They also have reported that plasma and cardiac renin activities were transiently elevated shortly after establishment of the shunt. Although we did not investigate the cardiac renin activity, there was no significant difference in cardiac or serum ACE activity 2 weeks after surgery. In our model, however, we cannot rule out the possibility that systemic or cardiac RAS is also activated transiently shortly after operation. According to the findings of Ruzicka et al.,321 the ACE inhibitor enalapril did not attenuate the increase in LV and RV weights in an aorticaval shunt model, despite the fact that ACE inhibitors have attenuated the increases in ventricular weights in AI models.17,18 These results provide further indications that there are differences between these two volume overload induced cardiac hypertrophy models.

In conclusion, chronic treatment with losartan attenuated LV hypertrophy in AI rats, and this effect was associated with a significant reductions in LV immunoreactive Ang II content. Losartan did not decrease PRA and plasma Ang II concentration. These results indicate that cardiac Ang II, rather than circulating Ang II, may play, at least in part, a crucial role in LV hypertrophy via AT1 receptors in AI rats.

REFERENCES AND NOTES


