EXPERIMENTAL STUDIES

Comparable Effects of Angiotensin II and Converting Enzyme Blockade on Hemodynamics and Cardiac Hypertrophy in Spontaneously Hypertensive Rats

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Jiro Kubota, M.D. and Keishiro Kawamura, M.D.

Angiotensin-converting enzyme inhibitors may regress left ventricular hypertrophy (LVH) without decreasing blood pressure (BP). The aim of the present study was to compare the effects of low and high doses of lisinopril and the angiotensin II receptor antagonist TCV116 (TCV) on LVH and hemodynamics in spontaneously hypertensive rats (SHR). Lisinopril (0.5 and 3 mg/kg per day) and TCV (0.3 mg/kg per day) were given to 8-week-old male SHR daily for 2 weeks. Untreated SHR and Wistar-Kyoto rats (WKY) served as controls. Untreated SHR had a greater left ventricular (LV) weight than WKY (p<0.01). Lisinopril (3 mg/kg per day) decreased both LV weight and BP. Lisinopril (0.5 mg/kg per day) significantly decreased LV weight, but not BP. In contrast, although TCV significantly decreased BP, LVH was not suppressed. Renal blood flow (RBF) in untreated SHR was less than that in WKY (p<0.05), but was increased with either lisinopril (3 mg/kg per day) or TCV. Cardiac output was increased only in lisinopril (3 mg/kg per day)-treated rats (p<0.05). These findings suggest that factors other than afterload reduction play a role in the regression of LVH with lisinopril, whereas a longer duration of treatment and/or a higher dose may be necessary with TCV. Despite the decrease in BP, TCV normalized RBF in SHR, perhaps due to the blockade of renal angiotensin II.

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Although angiotensin converting enzyme (ACE) inhibitors cause regression of left ventricular hypertrophy (LVH) in hypertensive humans1,2 and animals3 – 10 the mechanisms underlying this process have not been fully clarified. Afterload reduction appears to be an important factor in this regression8,9. However, Linz et al reported11 that ramipril, at a dose that did not decrease blood pressure, reversed LVH in aortic-banded rats. This finding suggests that factors other than blood pressure reduction play a role in the regression of LVH by ACE inhibitors.

The presence of a renin-angiotensin system has been demonstrated in the heart12 – 14. Since angiotensin II causes myocardial hypertrophy independent of blood pressure12,15 and ACE inhibitors block cardiac angiotensin II production16. ACE inhibitors may reverse LVH by suppressing the cardiac renin-angiotensin system. Angiotensin II receptor antagonists may also be effective in reducing LVH, but it is not clear how effective they may be compared to ACE inhibitors.

Key words:
Angiotensin II
Lisinopril
TCV116
Left ventricular hypertrophy
Spontaneously hypertensive rat

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The duration of treatment is another important factor in the regression of LVH. Recent reports have shown that short-term treatment (3 weeks) using various antihypertensive agents regressed LVH with a reduction of blood pressure in spontaneously hypertensive rats (SHR)\textsuperscript{17}

In this study, we compared the effects of low and high doses of lisinopril on blood pressure and LVH in SHR. TCV116 was administered to compare the effects of an ACE inhibitor to those of an angiotensin II receptor antagonist. Cardiorenal hemodynamics were evaluated after 2 weeks of treatment.

**MATERIALS AND METHODS**

**Animals**

Twenty-eight male SHR and 7 male Wistar-Kyoto rats (WKY) were obtained from Charles River Breeding Laboratories (Atsugi, Japan) at 7 weeks of age. The rats were housed 2 to a cage and given standard rat chow and water ad libitum. They were maintained in a room at a constant temperature (20–22°C) and humidity (50–60%). Following a 1-week acclimatization period, SHR were randomly divided into 4 groups as described below. All procedures were in accordance with the recommendations of the Declaration of Helsinki and approved by the College Animal Use and Care Committee.

**Drug Administration**

Lisinopril, obtained from Merck Sharp & Dohme Research Laboratories (Westpoint, Pennsylvania, USA), was administered to rats in their drinking water. TCV116, obtained from Takeda Chemical Industries, Ltd (Osaka, Japan), was suspended in 2% gum arabic solution and administered by a gastric gavage once a day in the morning. SHR were divided into 4 groups: Group 1: untreated as control, tap water only (n=7); Group 2: lisinopril in drinking water (0.5 mg/kg per day) (n=7); Group 3: lisinopril in drinking water (3 mg /kg per day) (n=7); and Group 4: TCV116 (0.3 mg/kg per day) by gastric gavage (n=7). In addition, untreated WKY (n=7) were used as a normal pressure control. A preliminary experiment showed that lisinopril (3 mg/kg per day in drinking water) adequately decreased blood pressure, and TCV116 (0.3 mg/kg per day by gavage) decreased blood pressure acutely almost to the same extent as lisinopril (3 mg/kg per day). Treatment began at 8 weeks of age and continued for 2 weeks.

**Hemodynamics**

Blood pressure and pulse rate were determined, just before and 7 and 14 days after treatment, in conscious animals by the tail-cuff method (UR-1,000, Ueda Inc, Tokyo, Japan) after the rats were kept at 37°C for approximately 15 min. The mean of 3 measurements was used for the study. Body weight and appearance were monitored regularly. At 2 weeks, cardiac catheterization was performed under pentobarbital anesthesia (50 mg/kg of body weight, intraperitoneally) as described elsewhere\textsuperscript{18} A 2F catheter-tip micromanometer (Model PR-249, Millar Instruments, Houston, Texas, USA) was introduced through the right carotid artery into the ascending aorta and left ventricular (LV) cavity. Systolic and diastolic pressure, LV end-diastolic pressure (LVEDP), dp/dt\textsuperscript{max} and heart rate were recorded at a paper speed of 100 mm/sec on a multi-channel recorder (Rectigraph-8K, San-Ei Instruments, Tokyo, Japan). LVEDP was displayed at a high sensitivity on another channel. Mean arterial pressure and pulse pressure were calculated. Cardiac output was obtained by the thermodilution method\textsuperscript{18} using a computer (Cardiotherm 500, Columbus Instruments, Columbus, Ohio, USA). After baseline hemodynamic measurements, the LV catheter was replaced by a thermistor-tipped catheter, the tip of which was placed in the ascending aorta just above the aortic valve. Normal saline at room temperature was injected rapidly through the venous catheter (0.1 ml). The mean of 3 readings of cardiac output was determined. Cardiac index, stroke volume, stroke index, total peripheral resistance (TPR) and TPR index (TPRI) were obtained as follows:

\[
\text{Cardiac index (ml/min per kg)} = (\text{cardiac output \ (ml/min)} / \text{body weight}) \times 1000 \\
\text{Stroke volume (ml/min)} = (\text{cardiac output/heart rate}) \times 1000
\]

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TABLE I  DATA FOR BODY, HEART AND KIDNEY WEIGHT AFTER TREATMENT

<table>
<thead>
<tr>
<th></th>
<th>SHR untreated (n=7)</th>
<th>SHR Lisinopril 0.5 mg (n=7)</th>
<th>SHR Lisinopril 3 mg (n=7)</th>
<th>SHR TCV16 0.3 mg (n=7)</th>
<th>WHY untreated (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>230±8</td>
<td>234±8</td>
<td>230±7</td>
<td>232±9</td>
<td>256±10</td>
</tr>
<tr>
<td>LV wt/body wt (mg/g)</td>
<td>2.63±0.07</td>
<td>2.38±0.07**</td>
<td>2.26±0.04**</td>
<td>2.25±0.09</td>
<td>2.22±0.06</td>
</tr>
<tr>
<td>RV wt/body wt (mg/g)</td>
<td>0.76±0.07</td>
<td>0.74±0.05</td>
<td>0.75±0.03</td>
<td>0.76±0.03</td>
<td>0.77±0.03</td>
</tr>
<tr>
<td>Kidney wt/body wt (mg/g)</td>
<td>3.53±0.12</td>
<td>3.61±0.16</td>
<td>3.55±0.12</td>
<td>3.53±0.17</td>
<td>3.77±0.21</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD  **p<0.01, vs untreated SHR; LV, left ventricular; RV, right ventricular; wt, weight.

![Graph showing systolic blood pressure over time](image)

Fig. 1. Time course of blood pressure measured in conscious rats. Before treatment, blood pressure was significantly higher in SHR than in untreated WKY (○). Lisinopril (0.5 mg/kg per day) (●) slightly reduced blood pressure at 1 week, but did not suppress elevation of blood pressure at 2 week. Lisinopril (3 mg/kg per day) (■) and TCV116 (●) reduced blood pressure significantly throughout the treatment. Bars are mean ± SD  *p<0.05, **p<0.01 vs untreated SHR. (□)

Stroke index (ml/kg)=stroke volume/body weight
TPR (mmHg/ml per min)=mean arterial pressure/cardiak output
TPRI (mmHg/ml per min per kg)= (TPR/body weight) × 1000

Renal blood flow was obtained by laser-Doppler flowmetry using a probe (ALF 2100, Advance Co, Tokyo, Japan) placed on the left kidney.

All of the above measurements were performed 2 or 3 h after the administration of TCV116 (between 10:00 and 12:00 h).

Heart and Kidney Weight

Cardiac arrest was induced by iv injection of 2% procaine (2 ml). The heart was rinsed with saline solution and blotted dry. After careful removal of the atria and great vessels, right and left ventricular weight were measured. Both kidneys were removed and the mean weight was determined. Absolute and relative values (organ weight per body weight) were used in the evaluation.

Statistical Analysis

All values are means ± SD. The data were first compared by an analysis of variance. When significant differences were identified, Scheffe’s test was performed for multiple comparisons between groups. Differences were considered significant when P < 0.05.

RESULTS

Body, Heart and Kidney Weight

The body weights of 10-week-old SHR were similar (232±8 g) in the 4 groups (Table I). The body weight of untreated WKY (256±10 g) was significantly greater (p<0.01) than those of the SHR groups. The LV weight per body weight ratio was significantly lower (14%, p<0.01) in the rats treated with lisinopril 3 mg/kg per day than in untreated SHR, and the ratio was similar to that in WKY. Lisinopril 0.5 mg/kg per day also decreased LV weight (9.5%, p<0.01) compared to untreated SHR. TCV116 did not decrease LV weight. Right ventricular weight and mean kidney weight (Table I) were similar in each group.
TABLE II HEMODYNAMIC DATA

<table>
<thead>
<tr>
<th></th>
<th>SHR untreated (n=7)</th>
<th>Lisinopril 0.5 mg (n=7)</th>
<th>3 mg (n=7)</th>
<th>TCV116 0.3 mg (n=7)</th>
<th>WKY untreated (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AoS (mmHg)</td>
<td>182 ± 9</td>
<td>177 ± 7</td>
<td>149 ± 13**</td>
<td>163 ± 13**</td>
<td>123 ± 12**</td>
</tr>
<tr>
<td>AoD (mmHg)</td>
<td>145 ± 13</td>
<td>144 ± 6</td>
<td>118 ± 9**</td>
<td>136 ± 11</td>
<td>103 ± 13**</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>157 ± 11</td>
<td>155 ± 7</td>
<td>128 ± 10**</td>
<td>145 ± 11</td>
<td>109 ± 12**</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>403 ± 30</td>
<td>424 ± 26</td>
<td>394 ± 34</td>
<td>412 ± 18</td>
<td>389 ± 55</td>
</tr>
<tr>
<td>LVs (mmHg)</td>
<td>180 ± 10</td>
<td>174 ± 9</td>
<td>150 ± 14**</td>
<td>164 ± 13**</td>
<td>120 ± 13**</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.3 ± 0.5</td>
<td>3.2 ± 0.4</td>
<td>3.2 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>dp/dt (mmHg/s)</td>
<td>1692 ± 87</td>
<td>1624 ± 73</td>
<td>1420 ± 117**</td>
<td>1595 ± 124</td>
<td>1260 ± 122**</td>
</tr>
<tr>
<td>CI (ml/min per kg)</td>
<td>341 ± 39</td>
<td>342 ± 32</td>
<td>388 ± 37*</td>
<td>352 ± 22</td>
<td>369 ± 38</td>
</tr>
<tr>
<td>SI (ml/kg)</td>
<td>0.85 ± 0.13</td>
<td>0.81 ± 0.12</td>
<td>0.98 ± 0.14*</td>
<td>0.86 ± 0.11</td>
<td>0.94 ± 0.13*</td>
</tr>
<tr>
<td>TPRI (mmHg/ml per min per kg)</td>
<td>0.46 ± 0.04</td>
<td>0.45 ± 0.04</td>
<td>0.33 ± 0.03**</td>
<td>0.41 ± 0.04</td>
<td>0.30 ± 0.03**</td>
</tr>
<tr>
<td>Flow/BW (ml/min per kg)</td>
<td>119 ± 14</td>
<td>136 ± 12</td>
<td>150 ± 24*</td>
<td>149 ± 20*</td>
<td>158 ± 23*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. *p<0.05 and **p<0.01 vs untreated SHR

AoS, aortic systolic pressure; AoD, aortic diastolic pressure; MAP, mean arterial pressure; LVs, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; dp/dt, ratio of pressure change; CI, cardiac index; SI, stroke index; TPRI, total peripheral resistance index; Flow, mean renal blood flow; BW, body weight

Hemodynamic Data

Fig.1 shows the time course of systolic blood pressure in conscious rats. The blood pressure in 8-week-old SHR before treatment (183 ± 7 mmHg) was significantly higher than that in WKY (142 ± 6 mmHg). Lisinopril 3 mg/kg per day produced a sustained decrease in blood pressure throughout the treatment period. Lisinopril 0.5 mg/kg per day slightly reduced blood pressure at 1 week, but did not suppress the elevation of blood pressure at 2 weeks. TCV116 decreased blood pressure similar to lisinopril 3 mg/kg per day at 1 week, and although blood pressure was increased at 2 weeks, the level was still lower than that in untreated SHR and before treatment. Heart rate was similar in each group. Table II shows the hemodynamic parameters obtained during cardiac catheterization at the end of treatment. Lisinopril 3 mg/kg per day significantly decreased systolic, diastolic and mean aortic pressure as well as LV systolic pressure compared to those in untreated SHR. TCV116 significantly decreased aortic systolic pressure and LV systolic pressure, but did not decrease aortic diastolic pressure. Therefore, the mean aortic pressure did not decrease. Lisinopril 0.5 mg/kg per day did not decrease any of these parameters. In SHR treated with lisinopril 3 mg/kg per day, the cardiac index was significantly increased, and the total peripheral resistance index and dp/dt\text{max} were significantly decreased compared to those in untreated SHR.

Mean renal blood flow per body weight obtained by laser-Doppler flowmetry was significantly decreased in untreated SHR compared to that in WKY (Table II). Renal blood flow was significantly higher with lisinopril 3 mg/kg per day and TCV116 than in untreated SHR.

DISCUSSION

In the present study, we compared the effects of different angiotensin II blocking agents on hemodynamics and cardiac hypertrophy in SHR. Previous investigations have demonstrated a regression of hypertrophied left ventricular mass in SHR with various antihypertensive drugs.\textsuperscript{8-17} ACE inhibitors effectively prevent cardiovascular hypertrophy with just a brief period of exposure.\textsuperscript{8-17}

Short-term treatment with lisinopril 3 mg /kg per day for 2 weeks inhibited the progression of hypertension and suppressed the development of LVH in SHR. Treatment with lisinopril 0.5 mg/kg per day for 2 weeks suppressed the development of LVH in SHR without reducing blood pressure. LVEDP

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and the total peripheral resistance index were similar between lisinopril (0.5 mg/kg per day)-treated and untreated SHR, indicating that the pre- and afterload in the left ventricle were similar. This suggests that factors other than afterload reduction play a role in the suppression of LVH.

The regression of LVH by ACE inhibitors may involve several factors. Afterload reduction due to vasodilation has been thought to be the most important factor. Neurohumoral effects, including the renin-angiotensin system and the sympathetic nerve system, should also be considered. Robertson and Khairallah reported that angiotensin II induced cardiac hypertrophy without increasing blood pressure in SHR. Recent examinations have demonstrated the existence of independently functioning, local renin-angiotensin systems in various extrarenal organs, such as the heart and blood vessels, suggesting that ACE inhibitors may have direct cardiac effects. Since angiotensin II has been implicated in increased protein synthesis in myocardial cells blockade of the cardiac renin-angiotensin system may contribute to the reversal of LVH. This speculation has recently been substantiated by Linz et al. who showed that ramipril reversed LVH without decreasing blood pressure in rats with aortic banding.

ACE inhibitors may also suppress the secretion of norepinephrine. A low dosage of norepinephrine which is insufficient to elevate blood pressure has been reported to induce LVH. Thus, LVH regression by a low dosage of lisinopril (0.5 mg/kg per day) in our study may be due to the suppression of angiotensin II and norepinephrine in cardiac muscle.

TCV116 0.3 mg/kg per day suppressed hypertension, but did not suppress LVH development. There are several possible explanations for this finding: 1) Lisinopril was given in the drinking water, while TCV116 was given by gastric gavage once a day in the morning. Continuous and stable blood pressure reduction over 24 h may not have been achieved in our TCV116-treated rats, as has been recently reported. 2) A dosage of 0.3 mg/kg per day might be too low to suppress LVH development with TCV116. In SHR, the most rapid increase in pressure is observed in young animals between the ages of 4 to 10 weeks, which has been considered to be the stage at which hypertension develops as shown in the present study. In our study, at 1 week, the blood pressure was suppressed to 160 mmHg by treatment with TCV116, which was similar to the suppression obtained by lisinopril 3 mg/kg per day. However, blood pressure had increased to 170 mmHg at 2 weeks, which was significantly less than that in untreated SHR, but higher than that at 1 week. Okada et al reported that 10 mg/kg per day of losartan, an angiotensin II receptor antagonist, decreased blood pressure and regressed LVH, but a dosage of 1 mg/kg per day did not. This finding may imply that TCV116 0.3 mg/kg per day was sufficient to reduce blood pressure due to vasodilation, which may be related to suppression of the vascular renin angiotensin system, but was not sufficient to suppress the local renin-angiotensin system in the myocardium, which seems to be related to LVH regression.

The treatment period is another important factor. Susic and Frohlich reviewed the effect of short-term (3-week) treatment using various antihypertensive agents in WKY and SHR. Many of these drugs reduced blood pressure and cardiac mass. However, the present 2-week treatment might be too short for TCV116 to induce LVH regression. The stress of gastric gavage once a day in SHR should also be considered.

Thus, afterload reduction appears to be more important in the regression of LVH by TCV116 than in that by lisinopril. Another factor to be considered is that ACE inhibitors, but not angiotensin II receptor antagonists, have been shown to stimulate the kinin-prostaglandin system. The enhanced kinins may contribute to the regression of LVH in our rats treated with a lower dosage of lisinopril. However, the present findings are too preliminary to draw a definitive conclusion regarding the different mechanisms for inducing regression with the 2 drugs.

Cardiac output was increased only by lisinopril 3 mg/kg per day. This effect may be due to afterload reduction through dilation of peripheral arteries by lisinopril, since heart rate and LVEDP (related to preload) were similar in each group.

Arendshorst et al reported that, compared

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to age-matched WKY, 4- to 6-week-old SHR are moderately hypertensive and have a reduced glomerular filtration rate and renal blood flow, and an increased renal vascular resistance. Observations from several laboratories support the notion that endogenous angiotensin II contributes to renal vasoconstriction in young SHR during the development of hypertension. Renal vascular tone in SHR has been reported to be more dependent on ACE activity than that in WKY. In our study, renal blood flow in untreated SHR decreased more than that in untreated WKY. However, renal blood flow in rats treated with lisinopril 3 mg/kg per day or TCV116 0.3 mg/kg per day was increased. This might be due to vasodilation of the renal artery caused by suppression of the local renin-angiotensin system in the kidney.

In conclusion, lisinopril decreased the blood pressure in SHR dose-dependently and suppressed the development of LVH even without decreasing blood pressure. This finding suggests that factors other than blood pressure reduction play a role in the regression of LVH by ACE inhibitors. TCV116 0.5 mg/kg per day decreased blood pressure, but did not regress LVH. Further studies, with a higher dosage and/or a longer treatment period, will be necessary to clarify the mechanism of the regression of LVH in SHR with TCV116.

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