PREVENTION OF NEPHROSCLEROSIS AND CARDIAC HYPERTROPHY BY CAPTOPRIL TREATMENT OF SPONTANEOUSLY HYPERTENSIVE RATS

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The relationship between hypertension and cardiovascular damage was assessed in three groups of spontaneously hypertensive rats (SHR): 1. stroke prone SHR (SHR-SP) treated orally with an angiotensin I converting enzyme inhibitor (captopril) (100–400 mg/L in the drinking water) from 6 to 35 weeks of age, 2. SHR-SP maintained on tap water until 30 weeks of age, 3. stroke resistant SHR (SHR-SR) maintained on tap water. The controls were Wister Kyoto rats (WKY) maintained on tap water.

Captopril-treated SHR-SP showed blood pressure lower than that of untreated SHR-SP, similar to SHR-SR. The ratio of heart weight to body weight was 0.55% in SHR-SP, 0.39% in captopril-treated SHR-SP, 0.46% in SHR-SR, and 0.39% in WKY. The kidneys of SHR-SP showed glomerular sclerosis, glomerular fibrosis, tubular casts, interstitial cell infiltration and vascular wall thickening or hyperplasia of the small arteries and arterioles. The severe glomerular sclerosis was mostly distributed in the inner and middle portions of cortex. Immunohistological study showed IgG, C3 and fibrinogen in the glomeruli and arterioles in SHR-SP. In captopril-treated SHR-SP, similar to SHR-SR, only minor histological changes were seen and there was no deposition of IgG, C3 or fibrinogen. No changes were seen in WKY.

Thus, it was concluded that nephrosclerosis and cardiac hypertrophy in SHR-SP are prevented by captopril. The role of the renin-angiotensin and kallikrein-kinin systems in organ pathogenesis in SHR-SP is discussed.

SPONTANEOUSLY hypertensive rats (SHR) develop various cardiovascular changes including cerebral hemorrhage and/or infarction, nephrosclerosis and cardiac hypertrophy. Stroke prone spontaneously hypertensive rats (SHR-SP) show an early rapid increase in blood pressure and develop severe hypertension (240–250 mmHg or more), while the highest blood pressure in stroke resistant SHR (SHR-SR) is about 200 mmHg.1

Cardiovascular lesions are more severe in SHR-SP than in SHR-SR. Hypertensive nephropathy has been considered to be the result of renal ischemia in severe hypertension, and cardiac hypertrophy in hypertension has usually been regarded as a secondary response to the increased pressure load. However, some discrepancies between the level of hypertension and the severity of cardiovascular lesions lead to questions as to whether high blood pressure is the only cause of the development of hypertensive vascular lesions?2 3 It has been reported that captopril prevents the development of both cardiac hypertrophy and hypertension in SHR4. In order to investigate

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140 Japanese Circulation Journal Vol. 50, February 1986
Effects of Captopril on SHR

Fig.1. a: Abundant fibrinoid and proliferative changes in arteries and arterioles and moderate glomerulosclerosis. (Masson trichrome x 200)
b: Severe glomerulosclerosis with PAS positive deposition in glomerulus and cyst-like tubule. (PAS x 200)

MATERIALS AND METHODS

Eleven SHR-SP, 6 SHR-SR, and 7 Wister Kyoto rats (WKY) were used. All rats were male and kept under the same conditions, properly housed, fed regular rat chow, and handled by the same person at fixed intervals. The animals were divided into the following 4 groups:
1) 5 SHR-SP maintained on tap water until 30 weeks of age,
2) 6 SHR-SP treated with captopril in drinking water (100–400 mg/L) from 6 to 35 weeks of age,
3) 6 SHR-SR maintained on tap water until 35 weeks of age,
4) 7 WKY maintained on tap water until 35 weeks of age.

Systolic blood pressure was measured by tail plethysmography of conscious rats warmed at 37°C for 8–10 minutes. The pressure was recorded once a week by the same person at approximately the same time of day. The rats were weighed once a week and immediately before they were killed. Protein concentration in the urine was determined one day before sacrifice by Albustix (Miles, Sankyo, Japan). SHR-SP given tap water were sacrificed at about 30 weeks of age. All rats were sacrificed by exsanguination from the abdominal aorta under ether anesthesia. Blood urea nitrogen (BUN) was measured by the urease-indophenol methods. The heart and kidneys were removed immediately, washed with saline, and weighed.

The kidneys were cut transversely at the midportion, fixed in Zenker formalin and embedded in paraffin for light microscopy.

whether captopril can prevent not only cardiac hypertrophy but also nephrosclerosis independently of blood pressure, we compared the cardiovascular lesions in SHR-SR and captopril-treated SHR-SP, in which the blood pressure was nearly the same.

Japanese Circulation Journal Vol. 50, February 1986
studies. Sections were cut and stained with hematoxylin-eosin (H.E.), periodic acid Schiff (PAS), Masson trichrome, periodic acid-methenamine silver (PAM), von Gieson and phosphotungstic acid hematoxylin (PTAH). Vascular changes were characterized by intimal thickening, onion skin appearance, and fibrinoid necrosis. We classified the glomerular lesions into four grades of severity, severe, moderate, mild and none (Fig. 1a, 1b). Severe glomerular lesions were hyalnosis, sclerosis, fibrosis and necrosis. Moderate glomerular lesions were mesangiosclerosis, periglomerular fibrosis and eosinophilic deposit in the tuft. Mild glomerular lesions were slight increase of mesangium and swelling of the Bowman’s capsule. The renal cortex was divided into three parts, the inner 1/3, middle 1/3 and outer 1/3. Injured glomeruli of untreated SHR-SP were counted in each part, where approximately 50 glomeruli were observed in each section.

For immunohistological studies of the kidneys, specimens were snap frozen in liquid nitrogen, sectioned in 2-μm thickness in a cryostat microtome and stained with peroxidase-labeled IgG antibodies (Fab’) against rat IgG, IgM, IgA, albumin, fibrinogen and C3 (Cappel Laboratories, U.S.A.). The specificity of these antisera was confirmed by immunoelectrophoresis and by immunodiffusion. Double-staining with PAS was performed after staining with peroxidase-labeled antibody.

Table I

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Body weight (g)</th>
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<tbody>
<tr>
<td>SHR-SP</td>
<td>5</td>
<td>281 ± 22.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>310 ± 14.9</td>
</tr>
<tr>
<td>SHR-SR</td>
<td>6</td>
<td>334 ± 46.9</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>374 ± 29.4</td>
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</table>

There were no significant differences among these rats.

Table II

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Ratio</th>
<th>Heart weight (g)</th>
<th>Significance</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Body weight (100g)</td>
<td>vs. WKY</td>
</tr>
<tr>
<td>SHR-SP</td>
<td>5</td>
<td>0.55</td>
<td>0.03</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.39</td>
<td>0.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>SHR-SR</td>
<td>6</td>
<td>0.46</td>
<td>0.02</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>0.37</td>
<td>0.01</td>
<td>—</td>
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n.s. = no significant difference; treated = treated with captopril

Table III

<table>
<thead>
<tr>
<th></th>
<th>BUN</th>
<th>Proteinuria</th>
<th>Renal lesions</th>
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<tr>
<td>SHR-SP</td>
<td>25.0</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>22.6</td>
<td>+++</td>
<td>—</td>
</tr>
<tr>
<td>SHR-SR</td>
<td>25.1</td>
<td>+++</td>
<td>—</td>
</tr>
<tr>
<td>WKY</td>
<td>19.5</td>
<td>++ — +</td>
<td>—</td>
</tr>
</tbody>
</table>

Proteinuria was determined by Albustix.
30 mg/dl < × < 100 mg/dl < +++ < 300 mg/dl < +++ < 1000 mg/dl < ++++
The degree of renal lesions was decided on the basis of renal glomerular injuries.

RESULTS

SHR-SP showed a rapid rise of blood pressure by 10 weeks of age and developed severe hyper-
Effects of Captopril on SHR

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>NUMBER OF SCLEROSED GLOMERULI IN ONE SECTION CUT TRANSVERSALLY AT MIDPORTION</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><strong>Cortical zones</strong></td>
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<td></td>
<td><strong>Inner</strong></td>
</tr>
<tr>
<td>SHR-SP 1</td>
<td>+ 15</td>
</tr>
<tr>
<td>SHR-SP 2</td>
<td>+ 10</td>
</tr>
<tr>
<td>SHR-SP 3</td>
<td>+ 24</td>
</tr>
<tr>
<td>SHR-SP 4</td>
<td>+ 18</td>
</tr>
<tr>
<td>SHR-SP 5</td>
<td>+ 16</td>
</tr>
</tbody>
</table>

Grade of severity:
+ = moderate injury (segmental glomerular sclerosis)
++ = severe injury (global glomerular sclerosis)

In the SHR-SP treated with captopril the blood pressure was lower than that in untreated SHR-SP, comparable to that in SHR-SR with one exception (Fig. 2), which showed blood pressure as high as that of untreated SHR-SP from 20 to 25 weeks of age. There were no significant differences in body weight among the 4 groups (Table I). Untreated SHR-SP, similar to SHR-SR, showed a significantly higher ratio of heart weight to body weight than WKY. In contrast, SHR-SP treated with captopril, similar to age-matched WKY, showed a significantly lower ratio than untreated SHR-SR (Table II).

The mean BUN levels were 25.0 ± 2.9 mg/dl in SHR-SP, 25.1 ± 1.0 in SHR-SR, 22.6 ± 7.0 in treated SHR-SP, and 19.1 ± 2.6 in WKY (Table III). BUN was higher in both SHR-SP and SHR-SR than in WKY but the differences were not significant. The BUN of treated SHR-SP, which ranged from 18.32 to 21.28 mg/dl except in one rat with a BUN of 36.6 mg/dl (heart to body weight ratio = 0.42), was lower than that of untreated SHR-SP, although statistically not significant (Table III). Severe proteinuria more than 300 mg/dl was observed in all SHR-SP, SHR-SR and treated SHR-SP rats whereas WKY showed mild proteinuria of less than 100 mg/dl (Table III).

There was no significant difference in kidney weight among 4 groups of rats (data not shown). When the renal histology was compared among these rats, morphological damage was not apparent in glomeruli or renal vessels of WKY. All untreated SHR-SP and one treated SHR-SP rat with high blood pressure from 20 to 25 weeks of age had severe kidney damage.

The glomerular changes in SHR-SP were characterized by the presence of glomerular sclerosis, which contained eosinophilic deposits. The number of segmentally sclerosed glomeruli ranged from 39 to 55 in each section containing approximately 150 glomeruli, and that of globally sclerosed glomeruli ranged for 2 to 29 in the same section. The other glomeruli without sclerosis were essentially normal by light microscopy, except for a slight increase of mesangium. Arterial changes, such as thickening of the wall and deposition of fibrinoid materials, were occasionally found in the interlobular arteries and afferent arterioles of untreated SHR-SP. Severely injured glomeruli coincided with

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Fig. 3ab. Immunodeposition of IgG in glomerulus and arteriole. (x 200)

fibrinoid sclerosis and onion skin appearance, and moderately injured glomeruli coincided with intimal thickening, while mildly injured glomeruli did not coincide with any distinct changes of vessels. The number of glomeruli with severe and moderate lesions was considered to be a histological index of renal lesions. Severe and moderate glomerular lesions were observed mostly in the inner 1/3 and middle 1/3 cortex (Table IV). Tubular casts were observed extending from the juxtamedullary portion to the superficial portion, occasionally without remarkable glomerular change. Injured tubules showed foci of thyroidization. The degree of tubular atrophy and interstitial fibrosis paralleled the severity of glomerular lesions. Severe and moderate changes of tubules, vessels and interstitium were also observed in the inner 1/3 and middle 1/3 of the cortex.

In SHR-SR and captopril-treated SHR-SP, with one exception, neither severe nor moderate lesions were found in the kidney, which exhibited only slight increase of glomerular mesangium.

In untreated SHR-SP, IgG, C3 and fibrinogen were found in the glomeruli, small arteries and arterioles (Fig. 3), while none was found in the glomeruli and vessels of the treated SHR-SP.

C3 was found non-specifically in the basement membrane of the tubules and Bowman's capsule in all rats. IgM was found in mesangium in some glomeruli and the interstitium in almost all rats.

DISCUSSION

The chronic administration of captopril in SHR-SP obviously decreases the ratio of heart weight to body weight, and at the same time prevents the development of malignant hypertension. Cardiac hypertrophy in hypertension has been considered to represent an adaptive response to chronically increased pressure overload. In the present study, however, there was no significant difference in blood pressure between captopril-treated SHR-SP and SHR-SR, whereas there was a significant difference in the ratios of heart weight to body weight between the two groups. This finding suggests the involvement of other factors in addition to arterial blood pressure in the development of cardiac hypertrophy associated with hypertension, supporting the hypothesis that the renin-angiotensin system may contribute to cardiac hypertrophy under certain conditions. In this context, consistent findings have been described by Sen et al. that α-methyldopa and hydralazine were equally successful in controlling hypertension, whereas cardiac ventricular weight is reduced by α-methyldopa but not by hydralazine, where plasma renin activity (PRA) is lowered by α-methyldopa but not by hydralazine.

Captopril inhibits not only the production of angiotensin II but also the degradation of bradykinin. Angiotensin II stimulates myocardial protein synthesis, modulates sympathetic nerve activity and inhibits norepinephrine reuptake. Accumulation of bradykinin in vascular smooth muscle is followed by enhanced synthesis of prostaglandins. Furthermore, prostaglandin E2 inhibits the norepinephrine release at the nerve endings. In addition, norepinephrine is considered to be responsible for translating physical stress to biochemical stimulation, resulting in increased myocardial protein synthesis. So, we suspect that captopril could prevent myocardial hypertrophy in SHR-SP by inhibiting the formation of angiotensin II and the degradation of bradykinin.

Administration of the β-adrenergic receptor blocking agents (propranolol or timolol) failed to alter either the naturally progressive rise of arterial pressure or the development of left ventricular hypertrophy. Some investigators have reported that propranolol is effective in reducing arterial pressure in the SHR while others reported that propranolol did not reduce blood pressure in SHR but somewhat reduced ventricular weight. Thus, the effects of β-adrenergic blockade are not uniform on arterial blood pressure and heart weight. Some kinds of β-adrenergic blockers suppress the action of the renin-angiotensin system, but not so completely as converting enzyme inhibitors. Enalapril, another angiotensin I converting enzyme inhibitor, prevented the development of hypertension and nephrosclerosis as well as cardiac hypertrophy in SHR-SP.

Although estimation of proteinuria did not reflect the effect of captopril in SHR-SP, the finding that tubular casts were found in only one treated SHR-SP suggests the involvement of arterial blood pressure in the leakage of protein urine.

The differences in BUN of the four groups were not significant, and not considered as a useful index of renal injuries in these rats, because even in SHR-SP, the number of sclerosed glomeruli was not high enough to cause severe
disturbance of renal function which is detected by increased BUN. The finding that severe and moderate glomerular sclerosis was mostly observed not in the outer but in the inner and the middle portion of the renal cortex in SHR-SP, suggests the involvement of local anatomic and physiologic factors predisposed to these glomerular scleroses. Captopril could prevent nephrosclerosis in SHR-SP by controlling hypertension, although the possibility that captoril improved blood distribution in the kidney, enhancing vascular dilatation by inhibiting the renin-angiotensin system and stimulating the kallikrein-kinin system, is suspected.

In malignant hypertension, the deposition of immunoglobulin and/or complement has been found in the vascular wall and an immunological mechanism of hypertensive nephropathy has been suspected. We examined the nephropathy of SHR by peroxidase labeled antibodies against these serum components. In untreated SHR-SP, deposits of IgG, C3, and fibrinogen in glomeruli and vessels were observed in focal distribution. However, these serum components were not found in treated SHR-SP and SHR-SR. These deposits are considered to be secondary to injuries of the vessels and glomerular capillaries caused by the progress of hypertension. Deposits of IgM occasionally seen in the glomeruli of almost all rats may be due to non-specific entrapment by mesangial cells because IgM has a large molecular weight.

Intravascular coagulation can play a part in the progress of hypertension. IgM, complement and fibrinogen are present in the injured vessels in malignant hypertension. Linton et al showed that local damage to arterioles was responsible for the initiation of intravascular coagulation with fibrin deposition and formation of microthrombi, suggesting that this narrowing of the vascular bed may play a role at the onset of malignant hypertension, presumably through activation of the renin-angiotensin system. In our study, IgG, fibrinogen and C3 were present in the small arteries and arterioles only in SHR-SP, suggesting that in severe hypertension, microcirculation of the kidney was changed, the walls of the vessels were injured by high blood pressure, and the deposits of IgG, fibrinogen and C3 accelerated the damage of the wall of the vessels.

In conclusion, the present study suggests that the administration of captopril in SHR-SP could, at least, prevent renal lesions, controlling hypertension and cardiac hypertrophy by not only controlling hypertension but also by enhancing vascular dilatation by inhibiting the renin-angiotensin system and stimulating the kallikrein-kinin system.

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