Chronic Effects of Enalapril on Blood Pressure, Stroke, Plasma Renin, Urinary Electrolytes and PGE_2 Excretion in Stroke-Prone Spontaneously Hypertensive Rats

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Accepted May 8, 1985

Abstract—Antihypertensive effect of enalapril (MK-421), an orally active non-sulfhydryl-containing converting enzyme inhibitor, was examined in stroke-prone spontaneously hypertensive (SHRSP) rats. The treatment was started at 14–15 weeks of age with tail blood pressure over 240 mmHg and was continued for 11 weeks. We used captopril as the reference drug. The dose of enalapril and captopril was 10 and 30 mg/kg per day, p.o., respectively. Enalapril showed a sustained antihypertensive effect from the 1st to the 11th week of the treatment. This antihypertensive effect was substantiated by the good increase in body weight; decrease in heart weight; decrease in incidences of vascular disease, nephrosclerosis, stroke and death. Enalapril treatment also prevented the increases in urine volume, and excretion of osmotically active solutes, Na, Cl and K with age. Captopril treatment showed about the same antihypertensive effect. No side effects were seen in the enalapril or captopril treated group. The antihypertensive potency of enalapril was about 3 times more than that of captopril. Enalapril and captopril slightly increased plasma renin concentration. Urinary excretion of PGE_2 was not changed by enalapril or captopril treatment. These results clearly demonstrate the efficacy of long-term treatment with enalapril to prevent development of malignant hypertensive cardiovascular disease in SHRSP rats.

Enalapril maleate is a new orally active converting enzyme inhibitor with no sulfhydryl group in its molecular structure (1, 2). Acute and chronic effect of enalapril on blood pressure (BP) has been examined in hypertensive animals, including spontaneously hypertensive rats (SHR) and renal hypertensive rats or dogs (3–8). However, no study has ever reported in stroke-prone SHR (SHRSP) rats. The antihypertensive effect of enalapril has also been confirmed in humans (9–11).

The purpose of the present study is to examine the antihypertensive effect of enalapril in SHRSP rats, as we did with captopril in previous reports (12, 13). Incidences of stroke, plasma renin, urinary electrolytes and PGE_2 excretion were also measured. As antihypertensive mechanisms of converting enzyme inhibitors are not fully elucidated (14, 15), it would be necessary to check parameters other than the renin-angiotensin system. A study on the kallikrein-kinin system in SHRSP rats was done in a separate series of experiments and will be reported elsewhere.

Materials and Methods

Hypertensive rats: Male SHRSP rats of 14–15 weeks of age were used. They were F62 from the colony of the Department of Pharmacology, Jichi Medical School. SHRSP rats had been transferred from the Biological Research Laboratories, Central...
Research Division, Takeda Chemical Industries at the F59 generation. Rats were housed five per cage in an animal room with constant temperature (25±1 °C) and humidity (55±5%), and automatic lighting cycle (7:00–19:00). They were given rat chow containing 0.18% (w/w) sodium and 1.13% (w/w) potassium (Nohsam MR-3-A) and tap water ad libitum.

Experimental design: Three experimental groups of 10 rats each were administered different combinations of drugs. The drug treatments were started at 15–16 weeks of age and were continued for 11 weeks, after a control observation for one week. The drugs were administered once a day by a gastric tube at 10:00–11:00 hours. Enalapril maleate (MK-421, Nippon Merck-Banyu) and captopril (Sankyo) were used. The drugs were dissolved with H2O in a volume of 5 ml/kg body weight. H2O (5 ml/kg) was given to the control group. The doses of enalapril and captopril were 10 and 30 mg/kg per day, respectively. The dose of enalapril referred to the free base. Means and standard errors are presented in the text, figures and tables. The significance of differences between mean values was evaluated by Scheffe’s S test. The x2-test, modified by Yates, was also used for statistical analysis of digital values. The correlation coefficient was calculated by the method of least squares.

Determination of blood pressure and heart rate: BP and heart rate (HR) were determined by a new model of a rat tail BP and HR determining system (Natsume Seisakusho KN-210) without anesthesia (16). This new system detects tail BP when the pulses disappear during the cuff inflation. The tail BP values determined by this new system is rather close to the maximum, but not to the mean BP obtained virtually by the conventional system (16). The rat was warmed for 3 min at 50°C before the determination. BP and HR were determined once a week immediately before treatment and for 10 weeks after the drug treatments had started. The interval between the drug administration and the determinations was 3–5 hr, and randomized in each rat. At the end of the drug treatments, mean BP was determined directly without anesthesia or restraint through a cannula which had been inserted into the abdominal aorta via the femoral artery 6–7 days before (17), by an electronic system (Century Technology CP-01, Star Medical PA-011, and Natsume KN-260). A good correlation between indirect tail and direct mean BP values was seen (r=0.943, P<0.01).

Postmortem examination: After the direct BP determination, 0.5 ml of blood sample was obtained from the aortic cannula into a syringe moistened with 15% EDTA diammonium solution. The rats were then anesthetized with ether and exsanguinated. The rats were inspected macroscopically, and the cranial cavity was opened to observe the brain surface. When both signs and abnormalities in the brain were observed, we counted that the rat had a stroke. The heart and kidney were weighed without fixation.

Determination of plasma renin concentration (PRC): The plasma was obtained by centrifuging the above blood sample at 3000 rpm for 20 min at 0°C. PRC measurement was conducted according to the modified method of Carvalho et al. (17). In brief, 50 μl of plasma sample was incubated for 2 hr with 200 μl of plasma renin substrate pool obtained from 24-hr-nephrectomized rats in the presence of inhibitors of angiotensinases and converting enzyme. The amount of angiotensin I generated during the incubation was determined by a radioimmunoassay. PRC was expressed as ng of angiotensin I formation per ml of sample plasma per hr.

Determination of urinary excretion of electrolytes and PGE2: Rats were housed one per metabolism cage (Natsume KN-646 (B-1)) and had free access to water and powdered food on the 3rd and 4th day and during the 5th and 10th week after the drug administration had started. Food and water intakes and urine volume were measured for 24 hr. Collected urine was frozen and stocked at -20°C until determination of urinary electrolytes. Osmolarity was measured by the freezing point depression using an osmometer (Fiske 130). Chloride (Cl) was measured by coulometric titration with a chloride meter (EEL). Sodium (Na) and potassium (K) were determined by a flame
Another 24-hr urine sample was collected into a reservoir cooled with dry ice. A one ml portion of the sample was lyophilized and subjected to an extraction procedure for PGE2. It was determined by radioimmunoassay with specific antiserum (Pasteur Institute, Paris, France) and tritiated PGE2 (New England Nuclear, Boston, U.S.A.). Urinary excretion of PGE2 was expressed as ng/kg per day.

Results

Body weight (Fig. 1): Body weight of the drug treated group increased to a greater extent than that of the control group. The differences as compared to the H2O group were statistically significant (P<0.05–P<0.01) from the 1st to the 11th week in the enalapril treated group and from the 2nd to the 11th week in the captopril treated group, respectively. Enalapril or captopril treatment improved the general condition of the rats during the experimental period. Only slight increases in food intakes were seen at the 3rd day and during the 5th week of experiments.

Blood pressure (Fig. 2): Tail BP of the control group continued to increase for 10 weeks from 249±6.6 mmHg to 274±6.4 mmHg. Mean BP determined directly at the 11th week was 243±8.0 mmHg. The difference between tail BP at the 10th week and mean BP at the 11th week was about 30 mmHg, approximately equal to 2/3 of pulse pressure in SHR rats (16). In the enalapril treated group, tail BP decreased markedly to 195±3.5 mmHg at the 1st week of the treatment. This decreased BP increased gradually toward the 10th week (224±6.1 mmHg), as seen in the H2O group. The BP differences from the H2O group throughout the experimental period were statistically significant (P<0.001). The captopril treated group showed almost the same BP pattern as that of the enalapril group. Tail BP at the 1st and the 10th week was 207±4.1 and 247±7.2 mmHg, respectively. The BP differences compared to the H2O group were statistically significant at the 1st–7th, 10th, and 11th week, respectively (P<0.05–P<0.01). The degree of BP decrease in the enalapril group was greater than that of the captopril treated group. However, the differ-
ence between the two groups was statistically significant (P<0.05) only at the 7th week.

Heart rate (Fig. 3): The control group showed fairly constant HR values (440–455/min) from the 1st to the 5th week, and they decreased gradually from the 6th week to the end of the experiment. This decrease was caused by 2 or 3 rats with lower HR values under 400/min because of severe hypertension and general weakness. HR values of the enalapril and captopril treated groups showed a tendency to increase from the 1st week compared to the H2O group and showed fairly constant HR values (460–

Fig. 2. Effects of enalapril and captopril given orally for 11 weeks on blood pressure in SHRSP rats. Tail BP was determined by the new model (Natsume Seisakusho KN-210). Mean BP was measured directly without anesthesia or restraint through a cannula inserted into the abdominal aorta. Details are the same as in Fig. 1.

Fig. 3. Effects of enalapril and captopril given orally for 10 weeks on heart rate in SHRSP rats. Details are the same as in Fig. 1.
480/min) until the 8th week, although HR tends to decrease (about 450/min) during the last 2 weeks; however, the difference was only statistically significant (P<0.05) at the 6th week.

Postmortem examination (Tables 1 and 2): The treatment with enalapril and captopril decreased heart weight significantly compared to the control group. Kidney weight of the captopril group showed significant decrease compared to the control. This was not seen in the previous study (13), and the reason is unknown. Kidney weight of the enalapril treated group also decreased slightly. In the control group, 5 rats had vascular lesion with polyarteritis nodosa in the mesenteric artery. No vascular lesion was found in the drug treated rats. Macroscopically observed nephrosclerosis was seen in the 5 rats of the H2O group. In these 5 rats, 4 rats showed accompanying vascular disease. Nephrosclerosis was not seen in the enalapril or captopril treated group. Eight rats had cerebrovascular stroke in the control group. The signs of stroke were seen at the age of 15–22 weeks of age. One rat showed stroke symptom before the experiment had started. Cerebral hemorrhage, and local softening of the brain were seen at the postmortem examination. No signs of stroke or abnormality of the brain surface were seen in the enalapril treated group. In the captopril treated group, one rat had softening of the brain with the signs of stroke, which had been seen before the treatment.

Survival rate was 50% in the control group. Two, one and two rats died at the 4th, 6th, and 11th week, respectively. All rats showed cerebral lesions and signs of stroke. In the enalapril and captopril treated groups, survival rate was 100%.

Plasma renin concentration (Fig. 4): The control value of PRC was almost the same as the previous study. The control value of PRC was almost the same as the previous study. The control value of PRC was almost the same as the previous study.

![Fig. 4. Effects of enalapril and captopril given orally for 11 weeks on plasma renin concentration in SHRSP rats. Vertical bars are the S.E. of the mean. No. of rats are at the bottom of the column.](image)

### Table 1. Heart and kidney weights in the SHRSP rats treated with enalapril and captopril

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart weight (g/100 g Body weight)</th>
<th>Kidney weight (g/100 g Body weight)</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>0.466±0.006</td>
<td>0.714±0.011</td>
<td>5</td>
</tr>
<tr>
<td>Enalapril</td>
<td>0.391±0.017*</td>
<td>0.694±0.008</td>
<td>10</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.381±0.011**</td>
<td>0.669±0.007**</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are the mean±S.E. *P<0.05 compared to the control. **P<0.01 compared to the control.

### Table 2. Incidences of vascular disease, nephrosclerosis, stroke and survival rate in the SHRSP rats treated with enalapril and captopril

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>Vascular disease</th>
<th>Nephrosclerosis</th>
<th>Stroke</th>
<th>Survival for 11 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Enalapril</td>
<td>10</td>
<td>0*</td>
<td>0*</td>
<td>0**</td>
<td>10*</td>
</tr>
<tr>
<td>Captopril</td>
<td>10</td>
<td>0*</td>
<td>0*</td>
<td>1*</td>
<td>10*</td>
</tr>
</tbody>
</table>

All figures indicate No. of rats. Data of the rats that died before the 11th wk of experiment were taken at the time of death. *P<0.05, compared to the control. **P<0.01, compared to the control.
as that in the previous report (17). PRC in the enalapril treated group increased twice compared to the H2O group. The difference was not statistically significant by Scheffe’s test, but significant (P<0.05) by the usual t-test. In the captopril group, PRC also increased only slightly as observed before (13), although the difference was statistically insignificant.

**Urinary excretion of water and electrolytes (Figs. 5–7):** Urine volume at the 3rd day and during the 5th and the 10th week are shown in Fig. 5. In the control group, urine volume at the 5th and 10th week was increased compared to the 3rd day, and this difference was statistically significant (P<0.05 and P<0.01, respectively). Water intake and excretion of osmotically active solutes were parallel with the urine volume in the control group. Enalapril and captopril treatments kept urine volume constant during the experimental period. Water intake and excretion of osmotically active solutes in the enalapril or captopril group were also kept constant almost parallel with urine volume (the data are not shown).

Urinary excretion of Na in the control group slightly increased at the 5th and 10th week compared to the 3rd day, but the difference was not statistically significant. In the captopril treated group, urinary excretion of Na slightly increased only at the 3rd day compared to the H2O group. In the enalapril treated group, Na excretion was almost the same as in the H2O group. CI excretion showed almost the same pattern as those of Na. At the 3rd day, captopril treatment increased it significantly (P<0.05). K excretion slightly increased during the 5th and 10th week in the control group. It was kept constant in the drug treated groups during the experimental period.

**Urinary excretion of PGE2 (Fig. 8):**

![Fig. 6. Effects of enalapril and captopril given orally for 10 weeks on urinary Na excretion in SHRSP rats. Details are the same as in Fig. 5.](image)

![Fig. 7. Effects of enalapril and captopril given orally for 10 weeks on urinary K excretion in SHRSP rats. Details are the same as in Fig. 5.](image)
Enalapril or captopril treatment had no effect on the urinary excretion of PGE2. However, PGE2 excretion decreased gradually with age. The differences from the 4th day were significant except enalapril group at the 5th week (P<0.01-0.05).

![Fig. 8. Effects of enalapril and captopril given orally for 10 weeks on urinary PGE2 excretion in SHRSP rats. Details are the same as in Fig. 5.](image)

Discussion

The antihypertensive effect of enalapril was compared with that of captopril in SHRSP rats. Both enalapril and captopril, 10 and 30 mg/kg per day, respectively, showed sustained decreases in BP, determined both indirectly and directly. The potency of the antihypertensive activity of enalapril may be three times more than that of captopril, as shown in the other hypertensive models in the previous reports (3-5).

We have improved the methods of direct and indirect BP determinations in this series of experiments. For taking BP indirectly we used a new model (Natsume Sessakusho KN-210) which detects BP during cuff inflation. It is usually determined during cuff deflation when blood flow in the tail artery reappears. This new device minimized the disturbances by animal movement and resulted in BP values near the maximum. We designated these indirect BP values as tail BP, as before. For the direct BP determination, we took a 6-7 day interval between the aortic cannulation and actual measurement, because we found that about a week is necessary for SHRSP rats to recover from surgery.

The above antihypertensive effect was substantiated by the following evidence obtained in this study: better increase in body weight, better survival rate, decreases in heart weight, lower incidences of vascular disease, nephrosclerosis and stroke, and prevention of increase in urine volume and urinary constituents with age. These changes in untreated SHRSP rats are characteristic in this type of hypertension model, as has been reported before (18, 19).

Increases in PRC after long term treatment of converting enzyme inhibitors in SHRSP rats were smaller, especially after captopril, than in the previous report (13). This was partly because the severity of hypertension in SHRSP rats previously used was less, and PRC remained relatively lower in the untreated control group. The greater increase in PRC with age in the present study made the difference between treated and untreated group smaller. Prevention of nephrosclerosis and sodium loss by the treatment was another factor which made the PRC differences smaller.

Swartz et al. suggested the role of PGE2...
in the BP lowering effect of captopril (20, 21). They observed the increase of blood PGE$_2$ metabolites after the acute treatment of captopril in humans. In SHR rats, urinary PGE$_2$ excretion was increased only at the 1st and 2nd day of captopril (100 mg/kg per day, p.o.) treatment during the 6 weeks of the experimental period (22). This decreasing effect of captopril on PGE$_2$ excretion was not seen after the 3rd day (22) and is in accord with the present observation after enalapril and captopril.

From the present studies, we can conclude that enalapril has an antihypertensive effect in SHRSP rats, and its potency is about 3 times more than captopril.

Acknowledgements: We thank Ms. Yuko Nakajima and Ms. Kuniko Haga for technical and secretarial assistances, respectively. Enalapril maleate was supplied by Dr. I. Ohmura, Pharmacology Laboratory, Nippon Merck-Banyu Co., Menuma.

References


