Beneficial Effects of Long-term Enalapril Treatment and Low-Salt Intake on Survival Rate of Dahl Salt-Sensitive Rats with Established Hypertension

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ABSTRACT
We investigated the effects of long-term treatment with the angiotensin-converting enzyme inhibitor enalapril and low-salt intake on the survival rate of Dahl salt-sensitive rats fed a high-salt (6.0% NaCl) diet. The systolic blood pressure of the rats increased gradually from 5 weeks of age and reached 240 mm Hg at 12 weeks of age. At this point, a low-salt diet group received a placebo (group 1, n = 10), and the high-salt diet group was divided into three groups: those given a placebo with the high-salt diet (group 2, n = 15), those given a chow change from a high- to a low-salt diet with a placebo (group 3, n = 14) and those given enalapril (30 mg/kg/day p.o., group 4, n = 14). At 19 weeks of age, all rats in group 1 were alive, and the survival rate of group 2 was only 40% (P < .001 vs. group 1). The survival rates of both groups 3 and 4 were significantly better: 86% (P < .01 vs. group 2) and 93% (P < .01), respectively. This beneficial effect on mortality was accompanied by an amelioration of the elevated plasma creatinine and urea nitrogen levels and a decrease in the glomerular sclerosis lesion scores in both groups. These results suggested that a high-salt content diet and the renin-angiotensin system are deterioration factors in lethal renal damage and the limitation of the diet salt content and inhibition of the renin-angiotensin system are important to improve the survival rate in high-salt-loaded hypertensive Dahl salt-sensitive rats.

In DS rats, high-salt loading for a long period causes progressive hypertension with renovascular (Sterzel et al., 1988) and/or cardiovascular diseases (de Simone et al., 1996; Inoko et al., 1994) and results in a short life span (Fleckenstein et al., 1987; Knorr et al., 1991). In contrast to the decreased survival rate of high-salt-loaded hypertensive DS rats, DS rats fed a low-salt diet were normotensive and survived longer (Inoko et al., 1994). Lee and Triggle (1986) showed that structural alterations of the arteries involved in the development of hypertension may be related to the amount of salt in the diet of DS rats. Accordingly, a high sodium intake is considered to be a critical factor of morbidity and organ damage in DS rats. Findings that the oral administration of an ACE inhibitor, captopril (Knorr et al., 1991), or an Ang II antagonist, losartan (von Lutterotti et al., 1992), before the onset of hypertension increased the life expectancy of DS rats fed a high-salt diet were normotensive and survived longer (Inoko et al., 1994). Lee and Triggle (1986) showed that structural alterations of the arteries involved in the development of hypertension may be related to the amount of salt in the diet of DS rats. Accordingly, a high sodium intake is considered to be a critical factor of morbidity and organ damage in DS rats. Findings that the oral administration of an ACE inhibitor, captopril (Knorr et al., 1991), or an Ang II antagonist, losartan (von Lutterotti et al., 1992), before the onset of hypertension increased the life expectancy of DS rats fed a high-salt diet revealed that the renin-angiotensin system is also an important factor in the end-organ damage. However, it is not yet clear whether the salt content and renin-angiotensin system affect the organ damage after the establishment of hypertension.

Methods

Animals. All experiments were carried out in accordance with our company’s guidelines for animal experimentation (Eisai Research Laboratories, Ibaraki, Japan).

Male DS rats at 4 weeks of age were purchased from BMW Laboratory (Gifu, Japan) and housed in a temperature- (23 ± 1°C) and moisture-controlled (55 ± 10%) room with a 12-hr light/dark cycle (lights on at 7:00 a.m. and lights off at 7:00 p.m.). The animals were fed a commercial diet (MF rat diet, Oriental Yeast Co., Tokyo, Japan), and tap water was freely given.

ABBREVIATIONS: DS, Dahl salt-sensitive; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; SBP, systolic blood pressure; HR, heart rate.
Experimental protocol. At 5 weeks of age, after measurements for SBP, HR and body weight, the rats were divided into a 0.3% NaCl (low-salt) diet group and a 6.0% NaCl (high-salt) diet group.

The experimental schedule is summarized in figure 1. The oral administration of enalapril or placebo was performed at 12 to 19 weeks of age. The animals fed the high-salt diet were divided into three groups at 12 weeks of age (groups 2–4).

Group 1 (n = 10) rats received only the low-salt diet for the entire course of the experiment and were administered a placebo (described below) daily from the age of 12 weeks. Group 2 (n = 15) rats continued to receive the high-salt diet and were also given the placebo.

Group 3 (n = 14) rats now received the low-salt diet in place of the high-salt diet and were given the placebo. Group 4 (n = 14) rats were maintained on the high-salt diet for the entire experiment and received enalapril (30 mg/kg/day) from the age of 12 weeks. The placebo (0.5% methylcellulose; Wako Pure Chemicals, Osaka, Japan) or enalapril (Sigma Chemical, St. Louis, MO) was orally administered once a day in a volume of 5 ml/kg.

Measurement of blood pressure. SBP and HR were measured by the tail-cuff method with a manometer-tachometer (KN-210; Nihon Kohden, Tokyo, Japan). The SBP and HR data are presented as the mean values of three consecutive measurements.

Mortality. The survival rates of the groups were monitored during the drug treatment period from 12 to 19 weeks of age and are expressed as the percentage of rats surviving within the groups.

Measurement of plasma biochemical parameters. At the end of the experiment (when the rats reached the age of 19 weeks), the rats were anesthetized with ether, and the chest and abdominal cavities were quickly opened. A blood sample was drawn from the abdominal artery and collected into a heparinized tube; it was then centrifuged at 4°C at 3000 rpm for 20 min to isolate the plasma. The concentrations of Na⁺, K⁺, Cl⁻ in the plasma were determined with an automatic electrolyte analyzer (model 710; Hitachi, Tokyo). The concentrations of blood biochemical parameters, such as total protein, albumin, total cholesterol, triglycerides, glucose, urea nitrogen, creatinine, glatamic pyruvic transaminase and glutamic oxaloacetic transaminases were determined with an autoanalyzer (model AU550; Olympus, Tokyo, Japan).

Measurement of heart weight. The heart was rinsed with ice-cold saline, and the atria and vessels were removed. The right ventricle free wall was separated from the left ventricle and septum for measurement of wet weight. The ratio of the heart weight to the body weight was calculated.

Histological study. The kidneys were fixed with a 10% formalin solution (pH 7.4; Wako) and embedded in paraffin for histological study. A minimum of 20 glomeruli in the plasma were determined according to the method of Luckhaus et al. (1982) (0 indicates no lesions; 1+, slight, lower degree and incidence of medial hyperplasia and peritubulitis; 2+, moderate, medial hyperplasia, adventitial fibrosis and peritubulitis; 3+, severe, numerous arteries demonstrating mainly necrosis, occlusion and fibrinoid). Tubular damage in both the medullary ray and inner stripe was graded from 0 to 4 according to the system of Uehara et al. (1991) (0 indicates no lesions; 1+, very mild focal dilation of tubules; 2+, larger number of dilated tubules with widening of interstitium; 3+, fairly extensive dilation of tubules with cystic formation and widening of interstitium; 4+, entire atrophy of tubules).

Drug. Enalapril maleate was suspended with 0.5% methylcellulose. The enalapril solution was prepared weekly and stored at 4°C.

Statistical analysis. Data are expressed as mean ± S.E.M. and were examined with a one-way analysis of variance. When a significant difference was detected, the data were further analyzed by Duncan’s multiple-range test and the Student’s t test. The survival rates are presented as Kaplan-Meier curves. The survival curves of individual groups were compared by the log-rank test and were analyzed further by using Dunnett’s multiple-range test. Values of P < .05 were considered statistically significant.

Results

SBP, HR and body weight changes. In the 5- to 12-week-old age period, the SBP of the DS rats fed the high-salt diet gradually increased and reached >240 mm Hg at 12 weeks of age.

Table 1 shows the group SBP, HR and body weight values at 12 and 19 weeks of age. At 12 weeks, SBP (P < .001) and HR (P < .01) were significantly high in the three high-salt-loaded groups (groups 2–4) compared with the continuous low-salt group (group 1).

In group 1, SBP and HR had not changed at 19 weeks of age, whereas in the high-salt-loaded DS rats (group 2), SBP (P < .001 vs. group 1) and HR (P < .01) continued to be significantly high. The chow change from high to low salt (group 3) and the enalapril treatment (group 4) did not decrease the elevated SBP. A body weight loss was observed in group 2, and the body weights in both groups 3 and 4 increased, similar to that in group 1.

Effects of diet change and enalapril on cardiac weight. The cardiac weights of the animals surviving at 19 weeks of age in the four groups are listed in table 2. Group 2 developed right (P < .01 vs. group 1) and left (P < .001) ventricular hypertrophy and cardiac hypertrophy (right ventricle and left ventricle plus septum, P < .001). In contrast, all of the indexes of cardiac weight were improved in the diet-change group (group 3), whereas the enalapril group (group 4) showed significant suppressions of both left ventricular (P < .05) and cardiac (P < .05) hypertrophy with no significant change in right ventricular hypertrophy.

Effects of diet change and enalapril on plasma biochemical parameters. The plasma biochemical parameters measured at 19 weeks of age are summarized in table 3. In the continuous high-salt group (group 2), the plasma total

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**Fig. 1.** Experimental design. MC, methylcellulose.
**TABLE 1**
SBP, HR and body weight in DS rats

<table>
<thead>
<tr>
<th>Group</th>
<th>12 Weeks old</th>
<th>19 Weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>1</td>
<td>144 ± 2.5</td>
<td>352 ± 6.9</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>248 ± 6.8b</td>
<td>429 ± 10.2a</td>
</tr>
<tr>
<td>(n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>244 ± 5.2b</td>
<td>444 ± 12.1a</td>
</tr>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>248 ± 6.3b</td>
<td>438 ± 8.7a</td>
</tr>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. Group totals are given in parentheses.

“p < .01 vs. group 1.

“P < .001 vs. group 1.

“P < .01 vs. group 2.

“P < .05 vs. group 2.

“P < .001 vs. group 2.

**TABLE 2**
Heart weight in DS rats at 19 weeks of age

<table>
<thead>
<tr>
<th>Group</th>
<th>RV/BW</th>
<th>LV + S/BW</th>
<th>RV + LV + S/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.43 ± 0.02</td>
<td>2.03 ± 0.03</td>
<td>2.47 ± 0.04</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.58 ± 0.04b</td>
<td>3.46 ± 0.18b</td>
<td>4.03 ± 0.20b</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.45 ± 0.01c</td>
<td>2.63 ± 0.03a</td>
<td>3.09 ± 0.03a</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.51 ± 0.02</td>
<td>2.96 ± 0.07c</td>
<td>3.47 ± 0.07c</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. Group totals are given in parentheses. RV, right ventricle; LV, left ventricle; S, septum; BW, body weight.

“p < .01 vs. group 1.

“P < .01 vs. group 1.

“P < .01 vs. group 2.

“P < .01 vs. group 2.

“P < .05 vs. group 2.

“P < .001 vs. group 2.

**Effect of diet change and enalapril on mortality.**

Figure 4 shows the Kaplan-Meier survival curves of each group. All DS rats in group 1 survived for the entire experimental period. The survival rate of group 2 decreased gradually to 40% at 19 weeks of age (P < .001 vs. group 1). Both group 3 and group 4 markedly improved the survival rate: 86% (P < .01 vs. group 2) and 93% (P < .01 vs. group 2), respectively.

**Discussion**

The major finding of this study was that both long-term (7-week) enalapril treatment and a chow change from a high- to a low-salt diet after the development of hypertension improved the survival rate independent of hypertension induced in DS rats by a high-salt diet. This beneficial effect of both treatments was accompanied by decreases in elevated plasma creatinine and urea nitrogen levels, an amelioration of glomerular sclerosis lesion scores and a suppression of cardiac hypertrophy.

In the present study, the chow change from the high- to the low-salt diet at 12 weeks of age did not alter the hypertension induced by the previous high-salt intake. In previous studies of DS rats, a high-salt intake for 7 or 8 weeks caused hyperplasia, arterial wall thickening and endothelial cell damage of the arteries (Lee and Triggle, 1986) and an attenuation of endothelium-dependent relaxation in the rats (Kitagawa et al., 1996). These results suggest that at this stage, impaired vasorelaxation may be developed as a result of a structural alteration of the arteries in DS rats; thus, we suspect that salt replacement fails to influence the structural alteration and hypertension. Along with the development of hypertension induced by a high-salt intake in DS rats, blood volume expansion is induced (Simchon et al., 1989), resulting in cardiac hypertrophy (Pfeffer et al., 1984). Therefore, a reduction in blood volume expansion by the low-salt diet may account for the suppression of cardiac hypertrophy observed in the present investigation.

Neither the chow change nor the inhibition of the reninangiotensin system influenced the hypertension, but each factor seems to have contributed to a decrease in mortality. Previous studies have reported that captopril and losartan improved the survival rate of DS rats with little effect on their hypertension (Knorr et al., 1991; von Lutterotti et al., 1992). These results indicate that in salt-loaded DS rats, some factor other than hypertension is important in the

cholesterol (P < .05), urea nitrogen (P < .05) and creatinine (P < .05) levels were significantly increased compared with group 1, indicating renal dysfunction. Group 3 showed significant attenuations of the elevations of all of the above plasma parameters compared with group 2. Group 4 also had ameliorated levels of plasma urea nitrogen (P < .05 vs. group 2) and creatinine (P < .05).

There were no significant differences in the plasma Na⁺, K⁺ or Cl⁻ levels at 19 weeks of age among the four groups (data not shown).

**Histological study.**

The glomerular sclerosis injury scores and renal arterial injury scores are summarized in figure 2. As shown in figure 2a, group 2 developed pronounced glomerular sclerosis lesions (75.0 ± 12.2, P < .01 vs. group 1). Both group 3 (8.4 ± 1.3, P < .01 vs. group 2) and group 4 (27.3 ± 2.8, P < .05) demonstrated a significant decrease in the glomerular sclerosis lesion score. The renal arterial damage (2.7 ± 0.3, P < .001 vs. group 1) was also increased in group 2 (fig. 2b). Although group 3 had significantly ameliorated renal arterial injury scores (0.4 ± 0.2, P < .001 vs. group 2), the scores of group 4 were not improved (2.5 ± 0.2).

The renal tubular injury scores in both the medullary ray (3.5 ± 0.3, P < .001 vs. group 1) and inner stripe (2.5 ± 0.3, P < .01) were increased in group 2 (fig. 3). Group 3 had lowered tubular injury scores only in the medullary ray (1.7 ± 0.2, P < .001 vs. group 2), not in the inner stripe (1.8 ± 0.2). Group 4 showed no improvement in tubular damage in either the medullary ray (2.9 ± 0.1) or inner stripe (2.1 ± 0.1).

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causation of lethal organ damage. One of the most impressive findings of our present study is that the chow change improved the renal dysfunction of hypertensive DS rats as shown by biochemical and morphological measurements. Accordingly, we believe that one of the factors in the mortality of DS rats is associated with salt-induced renal damage.

Enalapril delayed the mortality of stroke-prone spontaneously hypertensive rats, with a concomitant improvement in renal dysfunction (Stier et al., 1989). Losartan also decreased the mortality rate of high-salt-loaded DS rats with an improvement of renal lesion scores (von Lutterotti et al., 1992). Ang II plays a major role in the development of glomerular hypertension (Pelayo et al., 1990; Sterzel et al., 1988) and glomerulosclerosis (Anderson et al., 1993). The dosage of enalapril used in our experiment (30 mg/kg/day) is enough to inhibit the ACE activity in both the plasma and kidney (Unger et al., 1985). Therefore, we speculate that the inhibitory effect of enalapril on Ang II production in the kidney may contribute to the improvement of the renal damage and the survival rate.

Although it has been reported that antihypertensive drugs improve renal histological damage in DS rats (Uehara et al., 1991), the present findings revealed that a chow change reducing the salt content of the diet was more potent in reducing the renal morphological damage compared with the representative ACE inhibitor, enalapril. The beneficial effect of the chow change was confirmed by the marked amelioration of the renal arterial lesion scores. Indeed, the activation of the local renin-angiotensin system might play a major role in the development of renal damage after sustained hypertension was induced. However, further studies are necessary to clarify whether the salt content influences the local renin-angiotensin system.

It is known that long-term salt loading to DS rats induces a decreased sensitivity to beta adrenoceptor stimulation and a transition from cardiac hypertrophy to failing heart with diminished cardiac function (Inoko et al., 1994). These findings are similar to those in the myocardium from patients with heart failure (Böhmer et al., 1991). Enalapril has been shown to lengthen the life span of patients with heart failure in some clinical trials (The CONSENSUS Trial Study Group, 1987; The SOLVD Investigators, 1991). Our finding that enalapril treatment prolonged the long life span of DS rats is consistent with the results of these clinical trials. Our experimental procedure using DS rats may thus be an appropriate prospective approach for the pharmacological evaluation of drugs for the treatment of renovascular and/or cardiovascular diseases.

In conclusion, a high-salt intake and the renin-angiotensin
system play key roles in the development of end-organ renal damage, and the limitation of salt intake and inhibition of the renin-angiotensin system decreased the mortality rate of high-salt-loaded hypertensive DS rats.

References


