

## Influence of Imidapril on Abnormal Biochemical Parameters in Salt-Loaded Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

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**ABSTRACT**—Many of the disorders in urinary, biochemical, and hematological parameters induced by salt-loading in stroke-prone spontaneously hypertensive rats (SHRSP) were significantly ameliorated by chronic treatment with angiotensin converting enzyme inhibitors, imidapril (1 and 2 mg/kg) and enalapril (2 mg/kg). Through the improvement of these parameters, the treatment reduced the incidence of stroke but did not suppress the development of hypertension. These results suggest that the prophylaxis of stroke in SHRSP is probably due to systemic improvement as judged from the parameters of renal functions.

**Keywords:** Stroke-prone spontaneously hypertensive rats (SHRSP) (salt-loaded), Biological parameter (renal function), Imidapril

Some angiotensin converting enzyme (ACE) inhibitors, enalapril (1), captopril (2), delapril (3), and cilazapril (4), have been reported to exert a preventive effect on stroke in stroke-prone spontaneously hypertensive rats (SHRSP). The mechanism, however, is unclear.

Imidapril, a new ACE inhibitor, and enalapril equally lower blood pressure in spontaneously hypertensive rats (SHR) at more than 2 mg/kg, p.o. and in two kidney one-clip renal hypertensive rats at more than 0.5 mg/kg, p.o. (5). In SHRSP maintained on a 1% NaCl drinking solution, which is an accelerator of stroke incidence (6, 7), the preventive effect on stroke was not equipotent between the two agents. Imidapril at 0.5 mg/kg/day and enalapril at 2 mg/kg/day equally protected SHRSP from stroke occurrence to a certain extent, while imidapril at 2 mg/kg/day and enalapril at 5 mg/kg/day completely prevented stroke, although neither agent suppressed hypertension development (8).

Among the many antihypertensive agents, only some  $\beta$ -blockers are reported to reduce levels of blood urea nitrogen (BUN), creatinine (CRNN), phospholipid (PL) and triglyceride (TG) in SHRSP (9–11), while the effects of ACE inhibitors on these parameters in SHRSP have not yet been investigated.

In the present experiments, we examined: 1) the influ-

ence of salt-loading on various biochemical parameters in SHRSP and Wistar Kyoto (WKY) rats and 2) the effect of imidapril and enalapril on these parameters in salt-loaded SHRSP to investigate the mechanism of stroke prophylaxis.

Thirty-nine male SHRSP (obtained from Prof. Okamoto of Kinki University and bred at Tanabe Seiyaku Co., Ltd.) and 16 male WKY rats (Charles River Japan, Inc.) were maintained on a normal diet from 4 to 11 weeks of age, followed by a special diet (with high NaCl and low protein content; Funabashi-SP, Funabashi Farm Co., Ltd.) from 11 to 16 weeks of age. At 11 weeks of age, the SHRSP were divided into 2 groups based upon the systolic blood pressure as measured by the tail cuff method (BP monitor, MK-1000, Muromachi Kikai Co., Ltd.) and body weight. One group (n=8) was maintained on tap water ad libitum, as the non salt-loaded control. The other rats were given 1% NaCl solution instead of drinking water and subdivided into the 4 groups: control (n=7), imidapril (1, 2 mg/kg), and enalapril (2 mg/kg) (n=8 each). WKY rats were also divided into 2 groups (n=8 each) and served as the non-salt-loaded and salt-loaded groups. Imidapril hydrochloride and enalapril maleate (both synthesized at Tanabe Seiyaku Co., Ltd.) were dissolved in distilled water and given orally to the rats once a day for 5 weeks from 11 weeks of age. Systolic blood pressure was measured 24 hr after the 4th-week dosing. Neurological signs were observed daily (6). Body

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**Table 1.** The influence of chronic treatment with imidapril or enalapril on body weight, blood pressure, fluid intake, and urinary parameters of salt-loaded SHRSP

Breeding conditions	Drug (mg/kg)	N	Body weight (g)	Blood pressure (mmHg)	Fluid intake (ml/day)	Urinary parameters						
						Volume (ml/day)	Na (mEq/day)	K (mEq/day)	Cl (mEq/day)	Ca (mEq/day)	Protein (mg/day)	NAG activity (mU/day)
Salt-loaded	—	6	245.8 <sup>#</sup> ± 13.4	254.3 ± 12.8	56.0 <sup>#</sup> ± 9.8	51.9 <sup>###</sup> ± 6.6	9.18 <sup>##</sup> ± 1.30	1.00 ± 0.12	9.47 <sup>##</sup> ± 1.32	0.368 <sup>##</sup> ± 0.048	212.6 <sup>###</sup> ± 18.0	922 <sup>#</sup> ± 140
	Imidapril (1)	8	289.4 ± 8.8	272.7 ± 7.4	39.5 ± 8.8	29.4 ± 6.7	5.64 ± 1.29	0.99 ± 0.08	5.86 ± 1.33	0.161 <sup>**</sup> ± 0.035	69.4 <sup>**</sup> ± 23.9	460 <sup>**</sup> ± 37
	(2)	7	302.6 <sup>*</sup> ± 11.3	253.3 ± 1.6	30.0 ± 3.6	21.5 <sup>*</sup> ± 3.0	4.16 <sup>*</sup> ± 0.49	1.03 ± 0.07	4.41 <sup>*</sup> ± 0.52	0.106 <sup>**</sup> ± 0.037	30.2 <sup>**</sup> ± 2.4	404 <sup>**</sup> ± 27
	Enalapril (2)	8	288.8 ± 9.2	277.0 <sup>*</sup> ± 5.8	39.8 ± 6.1	31.0 ± 5.0	5.96 ± 0.95	1.14 ± 0.08	6.28 ± 0.98	0.196 <sup>*</sup> ± 0.038	74.0 <sup>**</sup> ± 20.9	491 <sup>**</sup> ± 52
Non-salt-loaded	—	8	295.3 ± 4.8	244.4 ± 4.8	20.4 ± 3.3	19.5 ± 3.5	0.77 ± 0.13	0.96 ± 0.08	0.91 ± 0.13	0.047 ± 0.008	40.5 ± 9.3	426 ± 61

NAG: *N*-acetyl- $\beta$ -D-glucosaminidase. Salt-loaded rats were maintained on 1% NaCl solution and a special diet (Funabashi SP), and non-salt-loaded rats were maintained on tap water and the special diet from 11 to 16 weeks of age. They were given the drugs orally once a day. After the final dosing, urine was collected for 24 hr. The contents of urinary electrodes and protein, and urinary NAG activity are expressed as excretion for 24 hr. Values are means with standard errors. \*:  $P < 0.05$ , \*\*:  $P < 0.01$  vs. salt-loaded SHRSP by Scheffe's multiple comparison, <sup>#</sup>:  $P < 0.05$ , <sup>##</sup>:  $P < 0.01$ , <sup>###</sup>:  $P < 0.001$  vs. non-salt-loaded SHRSP by Student's or Cochran's *t*-test.

**Table 3.** The influence of chronic treatment with imidapril or enalapril on the biochemical parameters of salt-loaded SHRSP

Breeding conditions	Drug (mg/kg)	N	GOT (mU/ml)	GPT (mU/ml)	GOT/GPT	LDH (mU/ml)	CPK (mU/ml)	K (mEq/l)	BUN (mg/dl)	CRNN (mg/dl)	TC (mg/dl)	TG (mg/dl)	PL (mg/dl)	HDLC (mg/dl)	HDLC/TC (%)
Salt-loaded	—	6	102.4 ± 7.6	41.2 <sup>##</sup> ± 4.3	2.69 <sup>#</sup> ± 0.43	475 ± 172	87.9 <sup>#</sup> ± 9.2	2.78 <sup>###</sup> ± 0.14	22.4 ± 4.0	0.66 <sup>##</sup> ± 0.06	130.5 <sup>###</sup> ± 11.5	154.0 <sup>##</sup> ± 17.4	244 <sup>###</sup> ± 18	77.4 <sup>##</sup> ± 7.1	59.4 <sup>##</sup> ± 2.0
	Imidapril (1)	8	81.6 <sup>**</sup> ± 1.6	47.0 ± 3.9	1.83 ± 0.16	139 <sup>*</sup> ± 26	68.0 ± 4.3	3.51 <sup>**</sup> ± 0.10	13.8 <sup>*</sup> ± 0.6	0.37 <sup>**</sup> ± 0.02	68.5 <sup>**</sup> ± 7.8	64.7 <sup>**</sup> ± 3.3	137 <sup>**</sup> ± 11	42.4 <sup>**</sup> ± 4.0	62.7 ± 2.1
	(2)	7	92.4 ± 3.5	57.3 <sup>*</sup> ± 3.4	1.63 <sup>*</sup> ± 0.06	138 <sup>*</sup> ± 20	63.5 <sup>*</sup> ± 5.8	3.44 <sup>**</sup> ± 0.05	12.0 <sup>**</sup> ± 0.5	0.31 <sup>**</sup> ± 0.01	54.8 <sup>**</sup> ± 1.0	55.7 <sup>**</sup> ± 3.9	114 <sup>**</sup> ± 1	36.2 <sup>**</sup> ± 1.7	66.1 ± 2.8
	Enalapril (2)	8	83.9 <sup>*</sup> ± 1.8	52.3 ± 1.9	1.62 <sup>*</sup> ± 0.08	111 <sup>*</sup> ± 6	59.6 <sup>**</sup> ± 2.4	3.27 <sup>*</sup> ± 0.08	14.4 <sup>*</sup> ± 0.7	0.34 <sup>**</sup> ± 0.03	62.8 <sup>**</sup> ± 8.7	62.2 <sup>**</sup> ± 7.0	132 <sup>**</sup> ± 11	43.9 <sup>**</sup> ± 4.3	72.6 <sup>*</sup> ± 2.9
Non-salt-loaded	—	8	88.4 ± 3.1	58.8 ± 3.4	1.52 ± 0.05	101 ± 5	59.3 ± 2.2	3.6 ± 0.08	16.9 ± 1.2	0.39 ± 0.02	50.1 ± 2.0	60.2 ± 2.4	115 ± 2	36.5 ± 1.3	73.4 ± 3.3

GOT: glutamic-oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, LDH: lactic dehydrogenase, CPK: creatine phosphokinase, K: potassium, BUN: blood urea nitrogen, CRNN: creatinine, TC: total cholesterol, TG: triglycerides, PL: phospholipids, HDLC: high density lipoprotein cholesterol. Salt-loaded rats were maintained on 1% NaCl solution and a special diet (Funabashi SP) and non-salt-loaded rats were maintained on tapwater and the special diet from 11 to 16 weeks of age. They were given the drugs orally once a day. Blood was obtained after the final dose following a 24-hr fast. Values are means with standard errors. \*:  $P < 0.05$ , \*\*:  $P < 0.01$  vs. salt-loaded SHRSP by Scheffe's multiple comparison. <sup>#</sup>:  $P < 0.05$ , <sup>##</sup>:  $P < 0.01$ , <sup>###</sup>:  $P < 0.001$  vs. non-salt-loaded SHRSP by Student's or Cochran's *t*-test.

weight was measured every day. After the final dosing, urine was collected, and water intake was measured during a 24-hr fast. Then blood was obtained from the abdominal aorta under ether anesthesia. Part of each blood sample was treated with EDTA·2K and used for hematological examination. The other part was treated with heparin-lithium and subjected to the measurement of electrolytes. Serum obtained from the residual blood was used to measure  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  levels, with an automated electrolyte analyzer (AIC PVA- $\alpha$ II). The erythrocyte (RBC) count, platelet (PLT) count, hemoglobin (Hb), hematocrit (Ht), and mean corpuscular volume (MCV) were measured with a blood cell counter (SYSMEX E-2500). Reticulocytes (Ret) were counted microscopically by brilliant cresyl blue staining. The GOT, GPT, LDH, and CPK activities and the BUN, CRNN, total cholesterol (TC), high density lipoprotein cholesterol (HDL), PL and TG levels in the serum and the *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) activity, protein, and  $\text{Ca}^{2+}$  levels in the urine were measured with an automated analyzer (TECTRON XA-18).

Statistical analyses were performed by Student's or Cochran's *t*-test for comparisons between the salt-loaded and non-salt-loaded groups, and Scheffe's multiple comparison test was used for assessing differences among the salt-loaded SHRSP.  $P < 0.05$  was considered to indicate a significant difference. One rat in the imidapril (2 mg/kg)-administered group never drank the drinking fluid in the sanitary cage even in the repeated trials; We omitted the rat from the experiment.

SHRSP with or without salt-loading equally developed severe hypertension; their systolic blood pressure was

$201.2 \pm 3.3$  mmHg at 11 weeks of age ( $n=39$ ). Blood pressure in the SHRSP treated with the ACE inhibitors was equal to or higher than that in the salt-loaded control group. Imidapril and enalapril increased the body weight, which was low in the control group, to the level in the non-salt-loaded group (Table 1). The blood pressure and body weight of WKY rats were not affected by salt-loading (data not shown).

In the salt-loaded control group, one animal died spontaneously, and 3 showed signs of stroke during the treatment period. One rat in the enalapril group also showed signs of stroke. The other rats survived without any evidence of stroke.

In SHRSP, water intake, urine volume, urinary  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ , protein excretion, and NAG activity were increased by salt-loading (Table 1). MCV and Ret were increased, while RBC, Hb, Ht, and PLT were decreased (Table 2). These changes were suppressed by imidapril in a dose-dependent manner. The extent of the changes by 2 mg/kg of enalapril was nearly equal to that by 1 mg/kg of imidapril (Tables 1 and 2). Urinary occult blood was found microscopically in the salt-loaded control SHRSP, but not in the SHRSP given ACE inhibitors (data not shown). The transaminase activity ratio (GOT/GPT) and the CPK, CRNN, TC, TG, PL, and HDL levels were increased, while the GPT,  $\text{K}^+$ , and HDL/TC levels were decreased in SHRSP by salt-loading. All of these changes in salt-loaded SHRSP were ameliorated by both ACE inhibitors equipotently (Table 3).

The values in the salt-loaded and non-salt-loaded WKY were  $38.1 \pm 5.3$  and  $25.8 \pm 0.9$  ml/day in fluid intake,  $29.4 \pm 5.4$  and  $20.8 \pm 1.1$  ml/day in urine volume,

**Table 2.** The influence of chronic treatment with imidapril or enalapril on the hematologic parameters of salt-loaded SHRSP

Breeding conditions	Drug (mg/kg)	N	RBC ( $10^7$ cells/ml)	Hb (g/dl)	Ht (%)	MCV (fl)	Ret (%)	PLT ( $10^7$ cells/ml)
Salt-loaded	—	6	$755^{\#\#}$ $\pm 54$	$13.0^{\#}$ $\pm 0.8$	$42.7^{\#}$ $\pm 2.2$	$57.0^{\#\#}$ $\pm 1.2$	$77^{\#}$ $\pm 18$	$38.1$ $\pm 5.0$
	Imidapril (1)	8	$951^{**}$ $\pm 21$	$15.8^{**}$ $\pm 0.3$	$49.0^*$ $\pm 0.8$	$51.6^{**}$ $\pm 0.3$	$33^*$ $\pm 4$	$46.6$ $\pm 3.6$
	(2)	7	$986^{**}$ $\pm 9$	$16.1^{**}$ $\pm 0.1$	$50.4^{**}$ $\pm 0.4$	$51.1^{**}$ $\pm 0.1$	$25^{**}$ $\pm 2$	$58.0^*$ $\pm 2.7$
	Enalapril (2)	8	$959^{**}$ $\pm 24$	$15.8^{**}$ $\pm 0.4$	$49.5^{**}$ $\pm 1.0$	$51.7^{**}$ $\pm 0.3$	$34^*$ $\pm 5$	$48.4$ $\pm 3.2$
	Non-salt-loaded	8	$992$ $\pm 7$	$16.2$ $\pm 0.1$	$50.3$ $\pm 0.3$	$50.8$ $\pm 0.2$	$26$ $\pm 2$	$50.4$ $\pm 3.6$

RBC: erythrocyte count, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, Ret: reticulocyte, PLT: platelet count. Salt-loaded rats were maintained on 1% NaCl solution and a special diet (Funabashi SP) and non-salt-loaded rats were maintained on tap water and the special diet from 11 to 16 weeks of age. They were given the drugs orally once a day. Blood was obtained from the abdominal aorta after the final dose following a 24-hr fast. Values are means with standard errors. \*:  $P < 0.05$ , \*\*:  $P < 0.01$  vs. non-salt-loaded SHRSP, by Scheffe's multiple comparison, #:  $P < 0.05$ , ##:  $P < 0.01$  vs. non-salt-loaded SHRSP, by Student's or Cochran's *t*-test.

$5.69 \pm 0.90$  and  $0.97 \pm 0.43$  mEq/day in urinary  $\text{Na}^+$ ,  $5.89 \pm 0.89$  and  $1.05 \pm 0.45$  mEq/day in urinary  $\text{Cl}^-$ ,  $0.078 \pm 0.01$  and  $0.042 \pm 0.005$  mEq/day in urinary  $\text{Ca}^{2+}$ , and  $11.2 \pm 0.4$  and  $13.5 \pm 0.7$  mg/dl in BUN, respectively. The values of urinary  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ , and BUN in salt-loaded WKY were significantly different from those in non-salt-loaded ones. The other parameters were not affected significantly by salt-loading.

Some  $\beta$ -blockers are reported to reduce levels of BUN, CRNN, TG, and PL in SHRSP due to the reduction of oxygen consumption based on the  $\beta$ -blockade (9–11).

Our results indicated that SHRSP are sensitive and WKY rats are resistant to salt-loading and that ACE inhibitors improve the disorders of biochemical parameters in salt-loaded SHRSP as  $\beta$ -blockers do.

In the salt-loaded SHRSP used here, imidapril and enalapril did not suppress the hypertension development. SHRSP is a low-renin type of hypertension model by nature. Salt-loading brings volume loading and additionally suppresses renin release from the kidney. Under these circumstances, the dose of 2 mg/kg is too low for imidapril and enalapril to exert an antihypertensive effect.

Some investigators have suggested that proteinuria was prominent in salt-loaded SHRSP (1, 3, 7). Reductions of proteinuria and urinary NAG activity in the SHRSP treated with ACE inhibitors may have resulted from the improvement of renal dysfunction as shown in this study. The changes of urinary parameters by salt-loading in WKY rats should be a result of their physiological responses. The remarkable increases in urine output and urinary  $\text{Na}^+$  and  $\text{Cl}^-$  excretion in SHRSP might have resulted from abnormal tubular reabsorption, because the changes were very markedly higher than those in WKY rats.

In the salt-loaded control SHRSP, the increases in BUN and CRNN and the hypokalemia suggested renal dysfunction. The decreases in RBC, Hb, and Ht and the increases in MCV and Ret probably resulted from kidney-related anemia upon loss of erythropoietin, because it is biosynthesized in the kidney and promotes synthesis of red blood cells in the marrow. Imidapril prevented the deterioration of all these parameters suggestive of kidney dysfunction. Stier et al. (1) reported that enalapril prevented proteinuria, improved the glomerular filtration rate, and reduced stroke in salt-loaded SHRSP. They also suggested a relationship between the amelioration of renal dysfunction and the prophylaxis of stroke. Our results support their suggestion on the basis of the biochemical parameters.

In salt-loaded SHRSP, the following changes were reported to occur in this order: 1) suppression of plasma renin activity, 2) histopathological disorders in renovasculature and elevation of urinary protein excretion,

3) elevation of plasma renin, and symptoms of stroke and histopathological disorders in cerebrovasculature (7). The amelioration of renal dysfunction could be a key to prevent stroke occurrence. A histopathological study was performed in the rats that survived in this study. All of the salt-loaded control SHRSP showed fibrinoid necrosis, glomerular sclerosis, and tubular degeneration in the kidneys, and half of them had small hemorrhagic foci in the brains. Even with salt-loading, a few renal changes and no cerebrovascular disorders were observed in the group given imidapril at 1 mg/kg. Imidapril at 2 mg/kg/day showed an excellent prophylactic effect on both the renal and cerebrovascular disorders. The data suggested that renal disorder predated cerebrovascular hemorrhage (12).

Reduction in PLT is reported to be a cause of additional cerebrovascular hemorrhage in SHRSP (13). The PLT count was perfectly recovered by imidapril. The cerebrovascular endothelium might remain intact in SHRSP treated with ACE inhibitors, since it is reported that the damage of the vascular lumen enhances a fall in the PLT count (14).

Cardiac abnormalities and elevated blood lipid levels were reported to be ingredients of the profile of stroke-proneness (15). The transaminase ratio, the levels of LDH and CPK, and blood lipid levels were decreased by both ACE inhibitors, and they seemed to protect the rats from heart disease. Moreover, the HDLC/TC ratio (an index of atherosclerosis) was also improved. This might be related to an increase in the  $\text{PGI}_2$  level that was augmented via the kinin-kallikrein-prostaglandin system by imidapril, because the compound is a vascular protective factor.

In conclusion, improvement of biochemical parameters, especially reno-functional parameters, by ACE inhibitors appears to relate to the preventive effect on stroke in salt-loaded SHRSP.

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