

ME3221, a Surmountable Angiotensin AT₁-Receptor Antagonist, Prevents Hypertensive Complications in Aged Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—The protective effects of ME3221, 3-methoxy-2,6-dimethyl-4-[[2'-(1*H*-tetrazol-5-yl)-1,1'-biphenyl-4-yl]methoxy] pyridine, on aged (32-week-old) stroke-prone spontaneously hypertensive rats (SHRSP) were studied following long-term (for 8 months) oral administration. At a dose of 10 mg/kg/day, ME3221 suppressed the mortality and the hypertensive complications observed in control SHRSP: cerebral apoplexy (hemorrhage, and spongeform and malacia in the cerebral cortex), increased proteinuria, and total *N*-acetyl- β -D-glucosaminidase activity, and cardiac hypertrophy and pleural effusion. The protective activity of ME3221, a surmountable angiotensin AT₁-receptor antagonist, was comparable to losartan, an insurmountable AT₁-antagonist, and also to enalapril, an angiotensin-converting enzyme inhibitor. In addition, ME3221 reduced the systolic blood pressure more effectively than the two reference drugs.

Keywords: ME3221, Angiotensin AT₁-receptor antagonist, Stroke-prone spontaneously hypertensive rat

Stroke-prone spontaneously hypertensive rats (SHRSP) are an experimental model of malignant hypertension with cerebrovascular disorders and end-organ damage (1). The aged SHRSP is believed to be a suitable animal model for studying the essential chronic hypertension in the elderly, since high blood pressure, high plasma renin activity and hypertensive complications are the main symptoms of aged SHRSP, different from spontaneously hypertensive rats (SHR). However, little work on aged SHRSP has been reported to date.

Losartan (DuP753), an angiotensin AT₁-receptor antagonist that has an antihypertensive effect (2, 3), was reported to prolong the survival of salt-loaded SHRSP (4–6). Losartan is a prodrug that is converted to the more active form EXP3174. EXP3174 is an insurmountable angiotensin AT₁-receptor antagonist and is a major contributor to the antihypertensive effect after the oral administration of losartan (7, 8). Thus losartan is regarded as an insurmountable angiotensin AT₁-receptor antagonist. The therapeutic efficacy of losartan has been reported to be equivalent to that of angiotensin-converting enzyme (ACE) inhibitors in clinical trials with the advantage of not inducing dry cough (9–11).

ME3221, 3-methoxy-2,6-dimethyl-4-[[2'-(1*H*-tetrazol-5-yl)-1,1'-biphenyl-4-yl]methoxy] pyridine (Fig. 1), is a surmountable angiotensin AT₁-receptor antagonist that

competes with angiotensin II in contracting the aorta in vitro (12) and has a potent antihypertensive effect in SHR and renal hypertensive rats (13, 14). EF2831, the major metabolite of ME3221 in humans, also shows surmountable angiotensin AT₁-receptor antagonistic activity (15).

The major purpose of this study was to examine the therapeutic efficacy of ME3221 by long-term administration against hypertensive complications of the aged SHRSP as a model of elderly chronic hypertension. Another purpose was to compare the long-term efficacy of surmountable and insurmountable angiotensin AT₁-receptor antagonists in this model. Wong et al. (7) speculated that an insurmountable antagonist has a longer-lasting effect than a surmountable antagonist. Thus, surmountable ME3221 and insurmountable losartan are appropriate candidates for a comparison of efficacy. We reported previously that ME3221 was three times more hypotensive than losartan against SHR by single administration (15) and prevented hypertensive complications in salt-loaded SHRSP by chronic administration (16), but no direct comparison of the two types of antagonists has been reported with respect to the long-term efficacy against hypertensive complications in non-salt-loaded SHRSP. Enalapril, which prevented stroke in salt-loaded SHRSP (17, 18), was used as a reference.

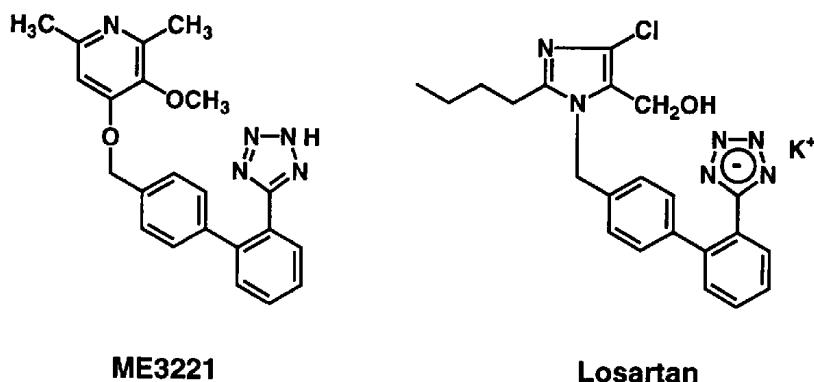


Fig. 1. Chemical structure of ME3221 and losartan.

MATERIALS AND METHODS

Male SHRSP (generations F-98, originally obtained from Prof. K. Okamoto, Kinki University, and bred in our laboratories thereafter) were used. The rats were initially fed a standard diet (0.31% Na and 29.1% crude protein content, NMF; Oriental Yeast Co., Ltd., Tokyo) and allowed water ad libitum. The room temperature was controlled at 22°C to 24°C, and a 12-hr light/12-hr dark cycle was maintained. When the rats were 32-weeks-old, they were randomized into four groups. The diet was replaced with a high Na and low protein diet for SHRSP (0.40% Na and 20.5% crude protein content, Funabashi-SP; Funabashi Farms Co., Ltd., Chiba). Salt (Funabashi-SP) was loaded to the animals from 32 weeks in order to prepare the long-life, aged SHRSP model. The four groups were treated as follows: the control group received 0.5% carboxymethylcellulose Na solution (2 ml/kg/day) (N=8), and the experimental groups received ME3221 (10 mg/kg/day) (N=8), losartan (10 mg/kg/day) (N=6) or enalapril (10 mg/kg/day) (N=8). All agents were administered orally once a day for 8 months. The systolic blood pressure (SBP) was measured once a week by tail-cuff plethysmography (PS-200; Rikenkaihatsu Co., Ltd., Tokyo) after prewarming the rats for 10 min at 37°C. The measurement was made about 3 hr after the drug administration as all the drugs showed almost the maximum depressive effect on SBP by oral administration (13). The body weight was measured at the same time. Since stroke causes depression of movement and eating, the systemic condition, neurological signs, and mortality were monitored daily. All the animals, including feeble and moribund rats during the drug administration period, that were alive after 8 months were killed by rapid decapitation for histological examination. Feeble and moribund rats were killed just before death to obtain organs for pathology inspection.

Urine examination

Rats were housed individually in metabolic cages (KN-647; Natsume, Tokyo) and allowed free access to rat chow and drinking water. The 24-hr urine samples were collected at 19 and 22 weeks after drug administration (51 and 54 weeks of age). The samples were centrifuged at 4°C at 3000 rpm for 10 min, and the supernatant was then stored frozen at -80°C until assay. The total *N*-acetyl- β -D-glucosaminidase activity (T-NAG), as an index of proximal tubule injury, and protein content in the supernatant urine were measured with an automated analyzer (COBAS FARA/FARA II; Roche, Basel, Switzerland).

Evaluation of organ weight and pathology

The brain, heart and kidneys were removed from each rat. The heart (ventricle) and kidneys (left and right) were weighed and expressed as g/100 g body weight, and the brain, excluding the olfactory bulb and cerebrum, was weighed and expressed in g. In the case of brain weight, compared with the other organs, the brain weight was weakly correlated with body weight. Then the organs were fixed in 10% formalin buffer and embedded in paraffin blocks. Histological sections from each block were stained with hematoxylin-eosin and then they were examined microscopically for lesions. The brain was sliced into 1-mm coronary slices, and the incidence and extent of hemorrhage, spongiform and malacia in the cerebral cortex and vitreous degenerated arterial wall were observed macroscopically or with a microscope. The scoring system for hemorrhage from the successive preparations were as follows: 1: none, 2: very slight (a minor lesion not definitely correlated with hemorrhage), 3: slight (diameter of hemorrhage: <0.5 mm), 4: moderate (diameter of hemorrhage: 0.5–1.5 mm), 5: severe (diameter of hemorrhage: >1.5 mm). The scoring system for the brain (except hemorrhage), heart and kidneys were as follows: 1: none, 2: very slight, 3: slight, 4: moderate, 5: severe.

Drugs

ME3221 and losartan were synthesized at the laboratories of Meiji Seika Kaisha Ltd. Enalapril maleate was purchased from Sigma (St. Louis, MO, USA). ME3221 was suspended in 0.5% carboxymethylcellulose Na solution, and the other drugs were dissolved in distilled water. The drug solutions were prepared every other day and administered in a volume of 2 ml/kg body weight.

Statistical analyses

All values except those on mortality are expressed as means \pm S.E.M. Statistical analyses were performed using analysis of variance and a multiple range test (Dunnett's test or Scheffe's test). Mortality was analyzed by the Kaplan-Meier method. The pathological findings were analyzed by the Kruskal-Wallis rank test and Mann-Whitney's *U*-test. Differences were considered significant at $P < 0.05$.

RESULTS

Effects on SBP

Figure 2 shows the time course of the effect of the drugs on SBP. Before drug administration (at 32 weeks of age), the SBP in all groups was around 240 mmHg. The SBP

in the vehicle-treated control rats rose to 255 mmHg at 35 weeks of age, and their SBP was maintained at about 250–260 mmHg during the experimental period, although it fluctuated greatly. ME3221 maintained the SBP at about 200 mmHg during the administration period, which was significantly ($P < 0.01$) lower than the blood pressure in the control rats. From 34 to 59 weeks of age, ME3221 lowered SBP significantly ($P < 0.05$ or $P < 0.01$). There was no tolerance to the long-term treatment. Both losartan and enalapril lowered SBP significantly ($P < 0.01$) compared to the control rats. From 35 to 59 weeks of age, losartan or enalapril lowered SBP significantly ($P < 0.05$ or $P < 0.01$).

There was a significant ($P < 0.01$) difference between ME3221 and the other two drugs in reducing SBP. Each data point of ME3221 was significantly ($P < 0.05$) different from that of losartan and enalapril at 40, 48 and 51 weeks of age and 34, 45, 46, 48, 50 and 51 weeks of age, respectively.

Effects on body weight, systemic symptoms and the survival rate

Body weight was measured weekly to monitor the incidence of stroke, and Figure 3 shows the changes. Although the change in body weight in all the groups was

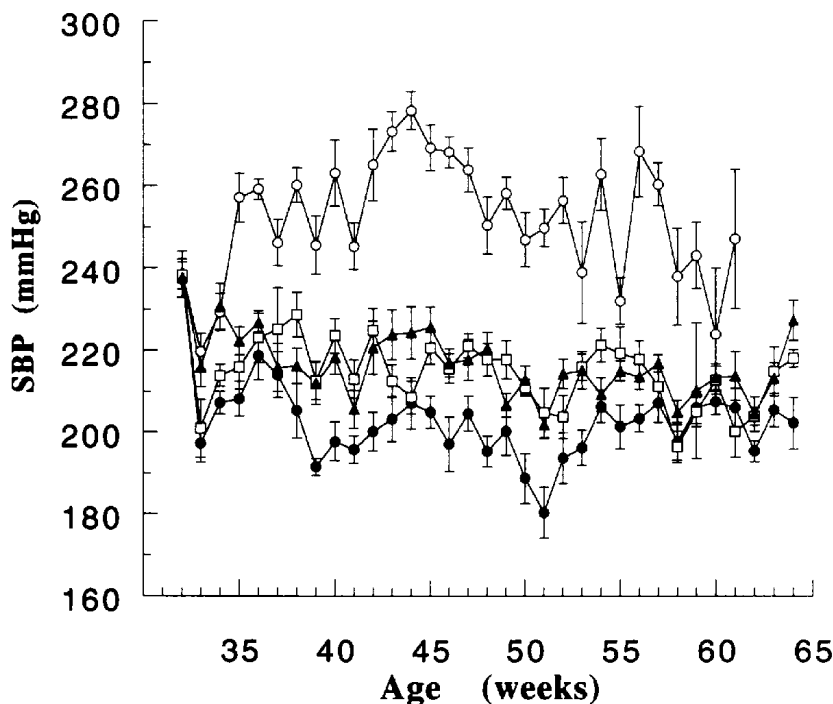


Fig. 2. Effects of ME3221, losartan and enalapril on systolic blood pressure (SBP) in stroke-prone spontaneously hypertensive rats (SHRSP). Drugs were administered orally to the rats from 32 to 64 weeks of age. \circ : control; \bullet : ME3221, 10 mg/kg; \square : losartan, 10 mg/kg; \blacktriangle : enalapril, 10 mg/kg. Each symbol represents the mean \pm S.E.M. of 6 to 8 rats. The SBP in the three drug groups were significantly different from that in the control at $P < 0.01$, and the SBP of ME3221 was significantly different from those of losartan and enalapril at $P < 0.01$ by Scheffe's test.

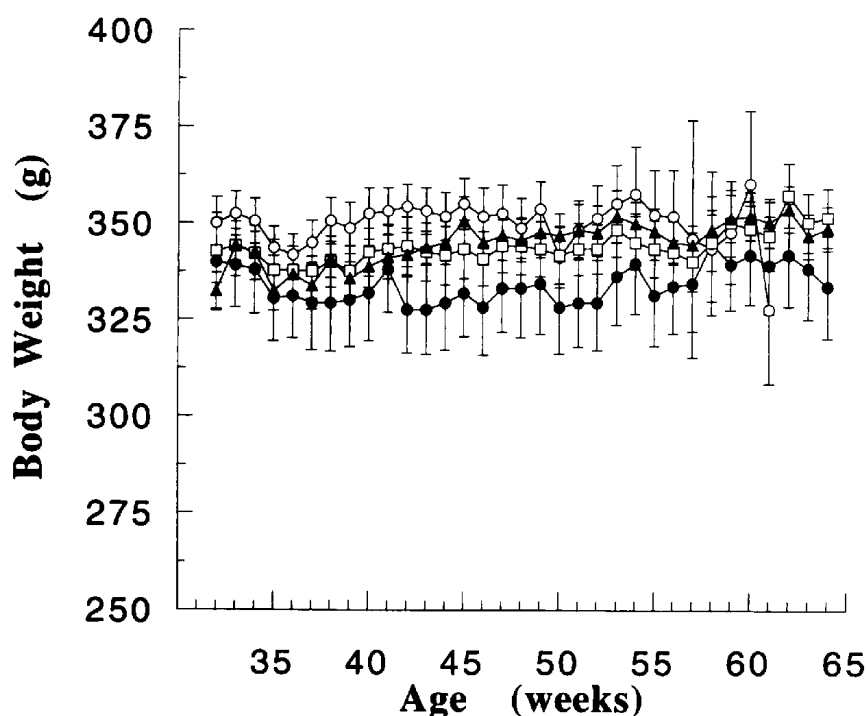


Fig. 3. Effects of ME3221, losartan and enalapril on body weight in SHRSP. Drugs were administered to the rats from 32 to 64 weeks of age. ○: control; ●: ME3221, 10 mg/kg; □: losartan, 10 mg/kg; ▲: enalapril, 10 mg/kg. Each symbol represents the mean \pm S.E.M. of 6 to 8 rats.

relatively small, the vehicle-treated control rats lost weight at 61 weeks of age, with a large standard for this value. The body weight in the three drug groups was not significantly different from that in the control group. From 50 weeks of age, 75% of the control rats showed ascites formation and systemic edema. However, the treatment with ME3221, losartan and enalapril did not produce these systemic symptoms.

Figure 4 shows the survival rate of the control and drug-treated SHRSP. In the control group, the first death occurred from 52 weeks of age, and all rats had died by 64 weeks of age. During the medication period, all rats treated with ME3221, losartan or enalapril survived, the survival rate in the treated groups being significantly ($P < 0.01$) higher than that in the control group.

Effect on hypertensive complications of brain

Figure 5 shows the brain weight of the SHRSP at 52 to 64 weeks of age. The brain weight in the control group was significantly greater ($P < 0.01$) than that in the rats at 32 weeks of age. Treatment with ME3221, losartan and enalapril significantly ($P < 0.01$) decreased the brain weight compared with that in the control group.

Table 1 shows the incidence and severity of cerebral hemorrhage in the SHRSP. The main site of hemorrhage was the cerebral cortex and basal ganglia. Most of the

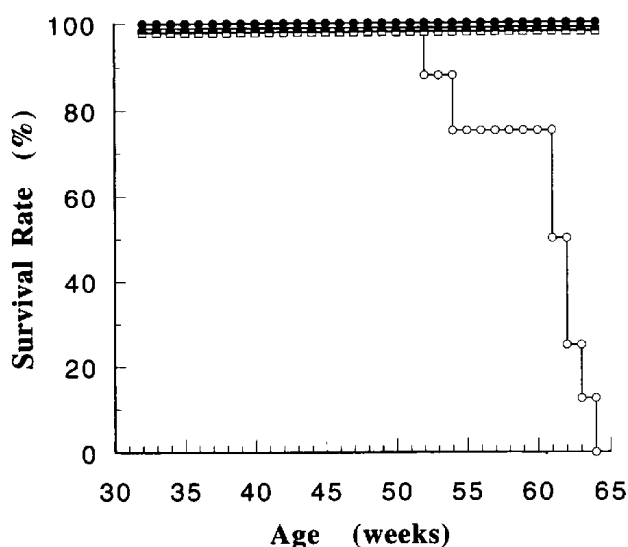


Fig. 4. Effects of ME3221, losartan and enalapril on the survival rate of SHRSP ($n=6-8$). Drugs were administered daily to the rats from 32 to 64 weeks of age. ○: control; ●: ME3221, 10 mg/kg; □: losartan, 10 mg/kg; ▲: enalapril, 10 mg/kg. The three drugs increased the survival rate in the SHRSP significantly compared to that in the control rats at $P < 0.01$ by the Kaplan-Meier method.

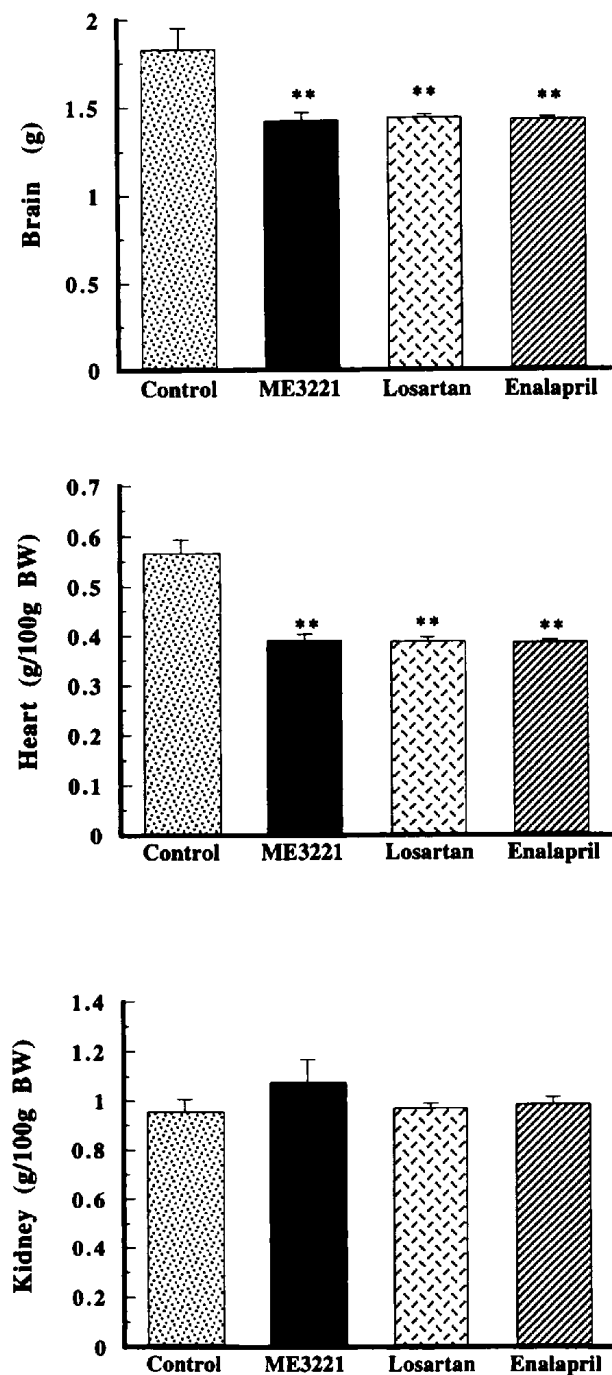


Fig. 5. Effects of ME3221, losartan and enalapril on organ weight in SHRSP. Each organ was isolated from SHRSP at 64 weeks of age in the treated groups and at 52 to 63 weeks of age in the control group. The weights of the heart (ventricle) and kidneys are expressed as g/100 g body weight (BW); and the weight of the brain, excluding the olfactory bulb and cerebrum, is shown in g. Drugs were administered orally to the rats from 32 to 64 weeks of age. Each column and bar represents the mean \pm S.E.M. of 6 to 8 rats. ** $P < 0.01$, as compared with the control group by the Dunnett multiple comparison test.

control rats at 52 to 63 weeks of age showed moderate cerebral hemorrhage. The mean scores of SHRSP treated with ME3221 were significantly ($P < 0.05$) protected from the cerebral hemorrhage. That is, two of the eight rats showed no hemorrhage, and the remaining six showed slight traces. Similar protection from hemorrhage was also observed in the rats treated with losartan and enalapril, although the hemorrhage scores in these two groups were not significantly lower than those in the control group.

The pathological examination of the brain of the control rats revealed the hemorrhage in small vessels, vitreous degenerated arterial wall, spongiform and malacia in the cerebral cortex. The treatment with ME3221, losartan and enalapril reduced these pathological changes (Table 1).

Aggression and hypersensitivity were found in more than half of the control rats from 54 weeks of age, but most of the neurological signs were temporary. Treatment with ME3221, losartan and enalapril did not produce the above neurological symptoms.

Effect on hypertensive complications of heart

The rats treated with ME3221, losartan and enalapril showed a significant ($P < 0.01$) reduction of the heart weight compared with the vehicle-treated control rats (Fig. 5). The three drugs suppressed cardiac hypertrophy. Moreover, pleural effusion, a sign of heart failure was found in the control rats, but not in the rats treated with any of the three drugs.

The control rats showed the following pathological signs: thickening and degeneration in the media of the coronary artery and hemorrhage and fibrosis around the cardiac ventricle cells. Treatment with ME3221, losartan and enalapril suppressed thickening and degeneration in the media of the coronary artery, but no difference was observed in the degree of improvement among the three drugs (Table 1, Fig. 6).

Effect on hypertensive complications of kidney

Figure 7 shows the effect of the drugs on urine volume, urinary protein and T-NAG in rats at 51 and 54 weeks of age. The urinary parameters in the rats treated with ME3221, losartan and enalapril were significantly ($P < 0.05$ or $P < 0.01$) lower than those in the control rats.

The kidney weight in the SHRSP treated with the three drugs was not significantly different from that in the control rats at 64 weeks of age (Fig. 5). Systemic edema, which is a sign of renal insufficiency was detected in the control rats, but not in the rats treated with any of the three drugs.

Pathological examination of the kidney specimen in the control revealed hypertrophy in the arterial wall, vitreous

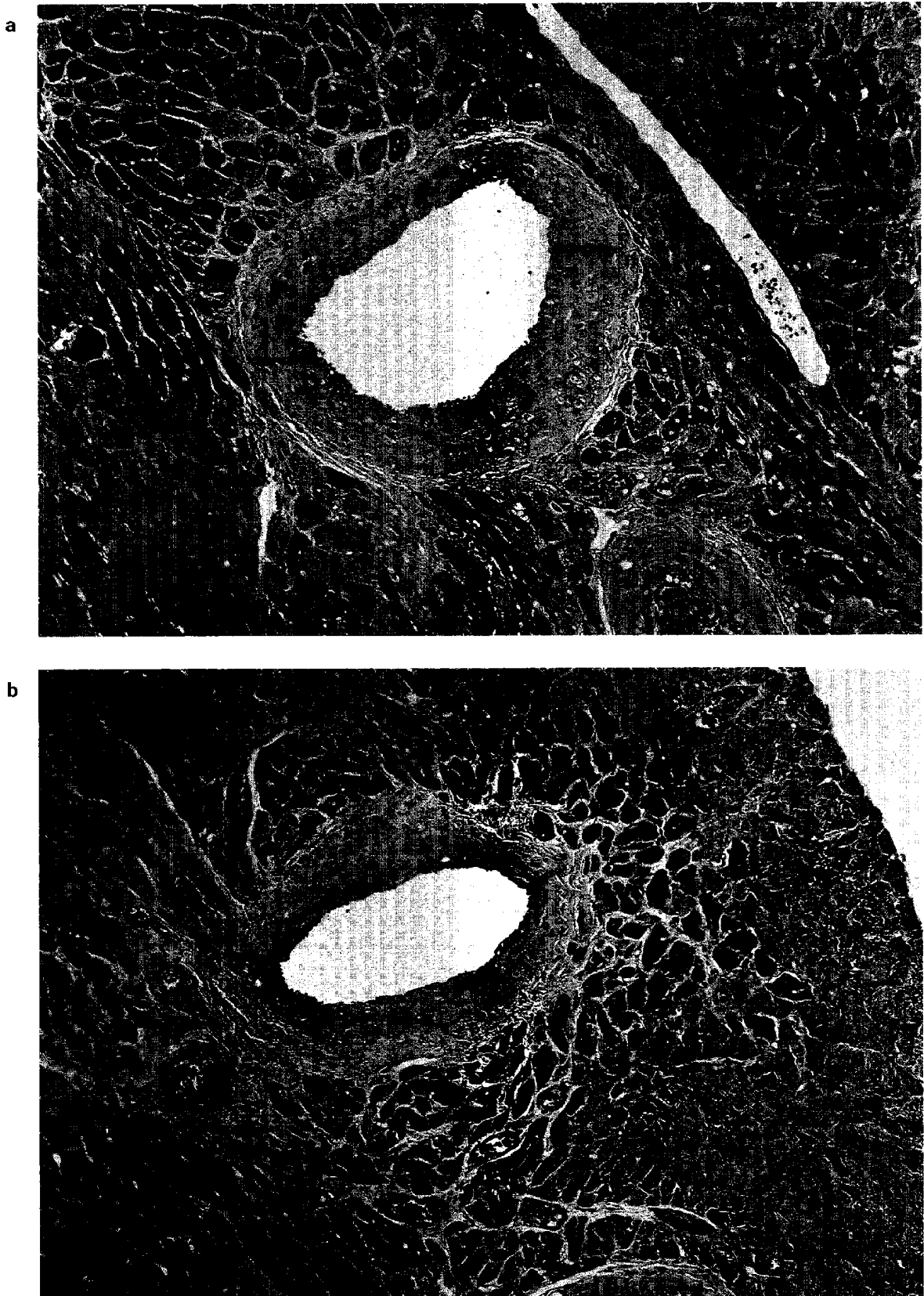


Fig. 6. Light microphotographs from the heart and kidneys of control and ME3221-treated SHRSP. a: Control (heart), b: ME3221 (heart), c: Control (kidney), d: ME3221 (kidney); hematoxylin and eosin stain, $\times 100$.

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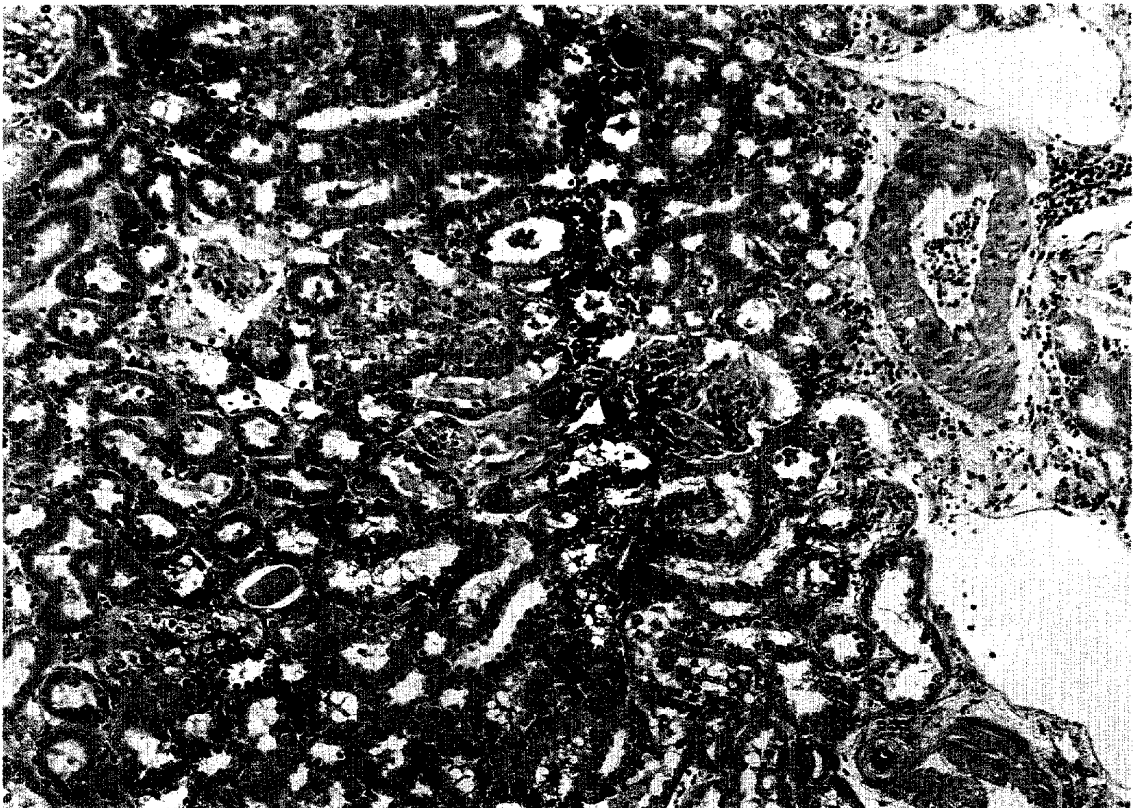


Fig. 6.

Table 1. Effects of ME3221, losartan and enalapril on the mean scores of pathological studies in SHRSP

Treatment	Control 0.5% CMC	ME3221 10 mg/kg	Losartan 10 mg/kg	Enalapril 10 mg/kg
Age (weeks)	52–63	64	64	64
N	8	8	6	8
Brain				
Hemorrhage [#]	3.38 ± 0.46	2.13 ± 0.30*	3.17 ± 0.31	3.13 ± 0.23
Spongiform and malacia in the cerebral cortex	2.88 ± 0.44	1.13 ± 0.13*	1.00 ± 0.00*	1.00 ± 0.00*
Vitreous degenerated arterial wall	2.13 ± 0.30	1.38 ± 0.18	1.17 ± 0.17*	1.00 ± 0.00*
Heart				
Thickening and degeneration in media of coronary artery	3.50 ± 0.19	2.75 ± 0.16*	2.67 ± 0.21*	2.88 ± 0.13*
Hemorrhage and fibrosis around the cardiac ventricle cells	3.25 ± 0.16	2.50 ± 0.38	3.00 ± 0.26	2.63 ± 0.18
Kidney				
Hypertrophy in the arterial wall	3.13 ± 0.13	1.25 ± 0.25**	1.00 ± 0.00**	1.13 ± 0.13**
Urinary cast	5.00 ± 0.00	3.38 ± 0.42**	3.83 ± 0.17**	3.75 ± 0.16**

Scores: 1: None, 2: Very slight, 3: Slight, 4: Moderate, 5: Severe. Drugs were administered to SHRSP daily from 32 to 64 weeks of age. N: Number of experimental animals. * $P < 0.05$, ** $P < 0.01$, as compared with the control group by the Kruskal-Wallis rank test and Mann-Whitney's *U*-test. Values are means ± S.E. [#]This score was obtained from successive preparations.

degenerated small arteries, hemorrhage and urinary cast. The pathological symptoms of nephrosclerosis were reduced by the treatment with ME3221, losartan and enalapril (Table 1, Fig. 6).

DISCUSSION

The aged SHRSP may be a moderate chronic model of hypertension, since it showed a gradual rise of the blood pressure to a level of 255 mmHg at 35 weeks of age, and was maintained at 250–260 mmHg up to 64 weeks of age. The control aged SHRSP gradually developed a stroke and died from 52 to 63 weeks of age. In contrast, the salt-loaded SHRSP may be regarded as a severe subacute model of hypertension, since it showed a rapid elevation of blood pressure up to 15 weeks of age to a maximum of 250–260 mmHg. The sudden death due to stroke occurred rapidly from 14 to 16 weeks of age (19). Although the clinical significance of these two models requires further investigation, it was of interest to compare the efficacy of ME3221, losartan and enalapril in these two models.

With regard to suppression of SBP elevation, the antihypertensive effect of ME3221 was significantly greater than that of losartan and enalapril. The higher efficacy of

ME3221 may be due in part to the more potent binding ability to AT₁ receptors as compared with losartan (15). More importantly, ME3221, losartan and enalapril completely protected the aged SHRSP from death up to 64 weeks of age.

The extended survival by ME3221 was closely related to the protection from cerebral hemorrhage, as shown in Table 1. Angiotensin II receptor antagonists and ACE inhibitors have been reported to reduce cerebral hemorrhage, which is considered to be a major life-threatening factor (5, 17, 18). Nagaoka et al. (19) reported that the incidence of stroke in SHRSP paralleled the increase in blood pressure, particularly when SBP was higher than 220 mmHg. Thus, the prolongation of survival with ME3221 may be associated with the antihypertensive action. However, Nagaoka (20) and Stier et al. (17) reported that non-hypotensive doses of ACE inhibitors and Ca²⁺ antagonists prevented stroke in SHRSP. These findings imply that the prevention of stroke depends not only upon lowering the blood pressure but also on some other factors, perhaps the inhibition of the renin-angiotensin system. In this respect, Nagaoka et al. (21) noted the importance of protecting the kidneys from ischemia in the prevention of stroke.

Regarding renal protection, long-term administration

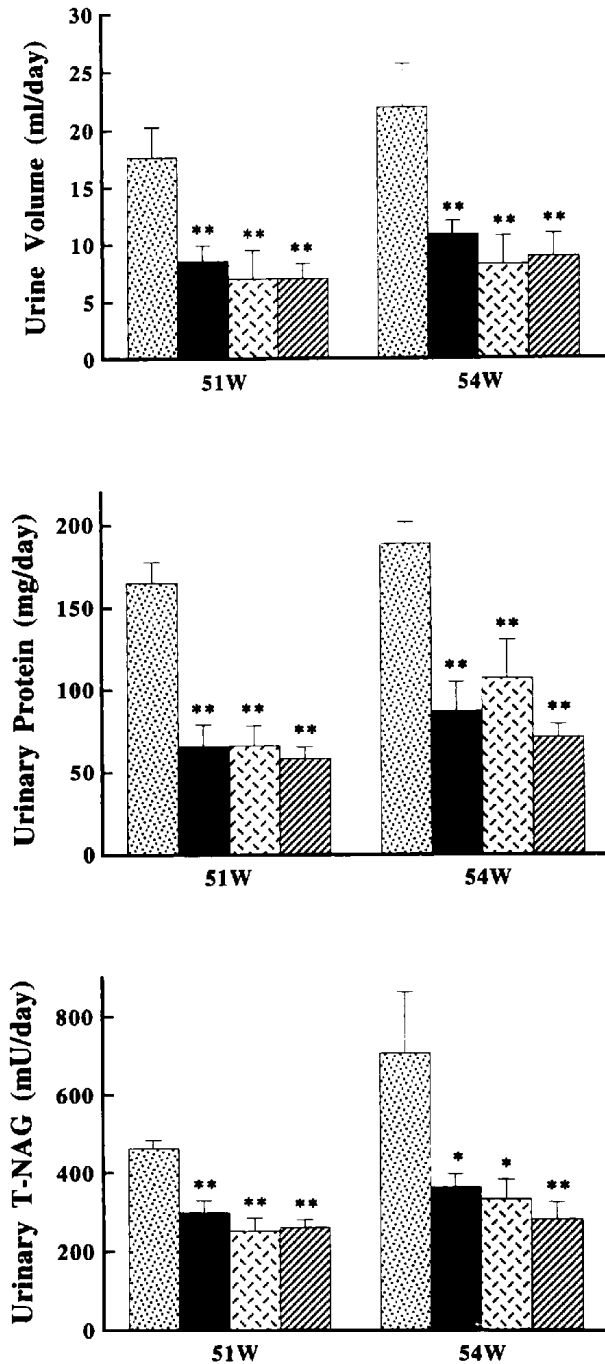


Fig. 7. Effects of ME3221, losartan and enalapril on urinary parameters in SHRSP at 51 and 54 weeks of age. Drugs were administered to the rats from 32 to 64 weeks of age. Each column and bar represents the mean \pm S.E.M. of 6 to 8 rats. \square : control; \blacksquare : ME3221, 10 mg/kg; \boxtimes : losartan, 10 mg/kg; \boxdot : enalapril, 10 mg/kg. * $P < 0.05$ and ** $P < 0.01$, as compared with the control group by the Dunnett multiple-range test.

of ME3221, as well as the reference drugs, markedly suppressed the progression of renal pathological changes, such as an increase of urine volume, proteinuria, T-NAG,

nephrosclerosis and systemic edema. Blantz et al. (22) reported that the glomerular filtration pressure was increased by angiotensin II and that the angiotensin II-induced contraction of the efferent arteriole was stronger than that of the afferent arteriole. ACE inhibitors not only lower the systemic blood pressure and inhibit angiotensin II-induced renal arterial contraction but also inhibit the reduction of glomerular filtration pressure, urine volume and the increase of proteinuria (23, 24). Losartan, an angiotensin II antagonist, has been reported to show this renal protection SHRSP (6) and hypertensive patients (25). Similarly, ME3221 may selectively prevent the contraction of the renal efferent arteriole and reduce the glomerular pressure for renal protection.

Previously, we found that there was no marked difference between surmountable and insurmountable AT_1 -antagonists on the antihypertensive effect, especially the duration of the effect by single administration (15) and also on the protective effect of hypertensive complications in salt-loaded SHRSP (16). Wong and Timmermans (8) reported that the insurmountable angiotensin II-antagonistic activity is reduced by the presence of surmountable AT_1 -receptor antagonist in an in vitro study. The surmountable AT_1 -receptor antagonist, losartan and insurmountable AT_1 -receptor antagonist, EXP3174 are considered to co-exist at an appropriate period after the administration of losartan, resulting in a weakening action of EXP3174 by losartan. A difference in the antihypertensive effect between ME3221 and losartan may be ascribed to the antagonistic effect seen in losartan. The cardiac hypertrophy of SHRSP has been explained in terms of the over stimulation of mechanical stretching due to hypertension and in terms of the action of biochemical factors, such as the angiotensin II-induced proliferation of cardiac myocytes and increased collagen synthesis (autocrine) (26), and the angiotensin II-induced release of norepinephrine from sympathetic nerve endings (paracrine) (27, 28). mRNA levels of angiotensinogen and ACE were shown to be increased in pressure-overloaded hearts in rats (29, 30). Hypertensive cardiac hypertrophy is related to the stimulation of the renin-angiotensin system in cardiac muscle tissue due to hypertension. The suppression of cardiac hypertrophy of ME3221 as well as losartan and enalapril is supposed to be the inhibition of this system. As reported separately (16), compared to the effect of enalapril, ME3221 and losartan showed superior protection against hypertensive complications in the salt-loaded SHRSP model. It may be of interest to see which model is most appropriate for estimating the clinical efficacy in the future.

In conclusion, our study indicates that long-term treatment with ME3221 prevented stroke and protected the kidneys due to a stable antihypertensive effect. With

regard to the antihypertensive effect and prevention of hypertensive complications, there was no definite difference between the surmountable angiotensin AT₁-receptor antagonist ME3221 and insurmountable antagonist losartan. These findings suggest that ME3221 should be of value in preventing progressive hypertensive complications in elderly patients.

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