Hypotensive Effect of SA446, an Angiotensin Converting Enzyme Inhibitor, in 2-Kidney, 1-Clip Renal Hypertensive and Normotensive Dogs

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Abstract—Hypotensive effects of SA446, an angiotensin converting enzyme (ACE) inhibitor, and effects on the renin-angiotensin system were evaluated in conscious normotensive and 2-kidney, 1-clip renal hypertensive dogs. SA446 (1 mg/kg, p.o.) remarkably inhibited the pressor response to angiotensin (Ang) I between 1 and 6 hr after the administration in normotensive dogs. SA446 significantly decreased blood pressure at 10 mg/kg, p.o., in normotensive dogs. During repeated administration of SA446 (100 mg/kg/day, p.o.) for 13 weeks, the blood pressure was lowered, and the pressor response to Ang I and plasma ACE activity were strongly inhibited. ACE activities in the aorta and kidney were also inhibited. Plasma renin activity and plasma Ang I concentration increased by repeated SA446 application, while plasma aldosterone concentration decreased. The hypotensive effect of SA446 (5 mg/kg, p.o.) was more potent in 2-kidney, 1-clip renal hypertensive dogs than in normotensive dogs. SA446 had longer inhibitory effects on the pressor response to Ang I and more potent hypotensive effects than captopril. The hypotension caused by SA446 appears to be associated mainly with an inhibition of ACE in plasma and also in the vascular wall.

It has been reported that SA446, a thiol compound similar to captopril, is a potent inhibitor of angiotensin converting enzyme (ACE), and the in vitro effect of SA446 is 4 to 5 times as potent as that of captopril (1). In anesthetized dogs, the inhibitory effect of SA446 on pressor response to angiotensin (Ang) I was about 3 times greater than that of captopril (1). However, the effect of SA446 was 2 times less potent than that of captopril in anesthetized rats (1, 2).

Recently, several orally-active inhibitors (1, 3–6) of ACE, including captopril, have been developed, and their effectiveness as antihypertensive drugs is established; however, the mechanism of the antihypertensive action of ACE inhibitors is controversial. Captopril lowers blood pressure in spontaneously hypertensive rats in which the plasma renin angiotensin system (RAS) does not appear to participate in the maintenance of high blood pressure (7), and an inhibition of plasma ACE activity does not always correlate with blood pressure reduction produced by ACE inhibitors (8–10). The hypotensive effect and the mechanism of action of ACE inhibitors have been analyzed mainly in rats (3, 5, 6), but not in dogs.

In the present study, oral effects of SA446 on blood pressure and RAS were evaluated in conscious 2-kidney, 1-clip renal hypertensive and normotensive dogs.

Materials and Methods

1. Drugs: SA446 [(2R, 4R)-2-(o-hydroxyphenyl)-3-(mercaptopropionyl)-4-thiazolidinecarboxylic acid] (Santen Pharmaceutical Co., Ltd., Osaka, Japan) and captopril were orally administered by capsules. Ang I, hippuryl-histidyl-leucine (HHL) and hippuric
acid were purchased from Protein Research Foundation, Osaka, Japan.

2. Inhibitory effect on pressor response to Ang I in conscious normotensive dogs: Mongrel dogs of either sex, weighing 8 to 15 kg, were anesthetized with intravenous injection of sodium pentobarbital (30 mg/kg) and a catheter (PE50) filled with heparinized saline was inserted into the carotid artery. After the one-day recovery period from the effect of the anesthesia, the catheter was connected to a pressure transducer (Nihon Kohden, MPU-0.5A). Intravenous injection of Ang I (0.1 μg/kg) was repeated at 30 min intervals through the cephalic vein until a constant pressor response was obtained. After the control response had been obtained, SA446 or captopril was administered orally, and the pressor response to Ang I was recorded at 30 min intervals for 6 hr.

3. Hypotensive effect of single SA446 administration in conscious normotensive dogs: Mongrel dogs of either sex, weighing 10 to 15 kg, were used for the study. After the dogs were trained enough to lie quietly on their left side on a table, the blood pressure was measured indirectly with a Programmed Electro-Sphygmomanometer (NARCO PE-300): a pressure cuff having microphone was placed around the right forelimb, and cuff pressure was raised to suprasystolic level and gradually reduced at a rate of 2 mmHg/sec. Cuff pressure and Korotkov’s sound waves were displayed on an ink-writing oscillograph (San-ei Instruments). The pressure corresponding to the first and final audible signals were regarded as the systolic and diastolic pressure, respectively. Blood pressure measurements were repeated several times, and the value obtained in the most quiet stable condition was counted. Mean blood pressure was calculated as follows: Mean blood pressure (mmHg) = diastolic pressure + (systolic pressure−diastolic pressure)/3

The blood pressure was measured for 6 hr after the oral treatment of SA446 at a dose of 10 mg/kg.

4. Effects of 13-week SA446 administration on blood pressure and RAS in conscious normotensive dogs: Experiments were performed in male beagles weighing 10 to 15 kg. SA446 at a dose of 100 mg/kg was administered orally once a day between 10 A.M. and 11 A.M. for a period of 13 weeks except Sundays. Before and 4 or 13 weeks (24 hr after the last drug administration) after long-term administration of SA446, blood samples were collected from the cephalic vein, and then the dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The animals were ventilated with room air by a respirator (Igarashi Ikakikai), and the arterial pressure was recorded by the pressure transducer connected to the catheter into the femoral artery. The pressor response to Ang I at doses of 0.05, 0.1 and 0.25 μg/kg, i.v., was also recorded. Furthermore, at the end of oral administration of SA446 for 13 weeks, the thoracic aorta and kidneys were isolated, and the ACE activities in these tissues were measured, compared with control beagles, according to the method described in the previous paper (11). Additionally, chronological changes in plasma ACE activity, plasma Ang I concentration, plasma renin activity (PRA) and plasma aldosterone concentration (PAC) during oral administration of SA446 at doses of 10, 30 and 100 mg/kg/day for 13 weeks were also determined.

5. Antihypertensive effect in 2-kidney, 1-clip renal hypertensive dogs: Mongrel dogs of either sex weighing 10 to 15 kg, trained well to lie quietly for the measurement of stable blood pressure as mentioned above, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the left renal artery was exposed via a retroperitoneal approach. The renal artery was constricted by a silver clip so that the blood flow measured by an electromagnetic flowmeter (Nihon Kohden, MFV-2100) was reduced to approximately 30–40% of the original flow. Two and four weeks after the constriction, SA446 at a dose of 5 mg/kg was administered orally, and blood pressure was measured indirectly.

6. Determination of plasma ACE activity, plasma Ang I concentration, PRA and PAC: Blood samples were collected into plastic tubes containing heparin (25 U/ml) or disodium EDTA (1 mg/ml) for determinations
of ACE activity or Ang I concentration, PRA and PAC, respectively. They were centrifuged for 10 min at 3000 rpm at 4°C to separate plasma. Immediately after the separation, plasma ACE activity was determined as the rate at which hippuric acid was released from HHL (synthetic substrate) by using a reversed phase high-performance liquid chromatography according to the method of Horiuchi et al. (12). One unit of ACE activity was defined as the amount of enzyme that generated 1 μmol of hippuric acid in 1 min at 37°C. Plasma Ang I concentration, PRA and PAC were determined by radioimmunoassay using CIS-CEA kits.

7. Statistical analysis: In the case of comparison of the control with two or more groups, one-way analysis of variance was performed, and significance of difference between them was evaluated by Dunnett's test. Significance of difference between two groups or intragroup was evaluated by Student's t-test or the paired t-test, respectively.

Results
1. Inhibitory effect on pressor response to Ang I in conscious normotensive dogs: Before the administration of inhibitors, the pressor response to Ang I did not differ between two groups for SA446 treatment (38±5 mmHg, N=5) and for captopril treatment (33±3 mmHg, N=5). Figure 1 shows the time course of inhibitory effects of SA446 (1 mg/kg, p.o.) and captopril (1 mg/kg, p.o.) on the pressor response to Ang I. SA446 significantly inhibited the pressor response between 1 and 6 hr after the administration. The maximum inhibition was observed at 3.5 hr (79% inhibition), and a 56% inhibition was observed even 6 hr later. Captopril significantly inhibited the pressor response between 0.5 and 4.5 hr with a maximum inhibition of 79% at 1.5 hr after the administration. However, the inhibition by captopril was reversed 5 hr later. SA446 inhibited the response to Ang I to a significantly greater extent at 3.5, 4, 5.5 and 6 hr than captopril.

2. Hypotensive effect of single SA446 administration in conscious normotensive dogs: Figure 2 shows the change in mean blood pressure following a single oral administration of SA446 (10 mg/kg) to conscious normotensive dogs. Before the drug administration (0 hr in Fig. 2), mean blood pressures in the control and SA446-treated group were 92±7 and 96±4 mmHg, respectively. Significant decrease of mean blood pressure was observed 2 hr after SA446 administration.

3. Effects of 13-week SA446 administration on blood pressure and RAS in con-
conscious normotensive dogs: Figure 3 shows changes in mean blood pressure and pressor response to Ang I. During the repeated administrations of SA446 (100 mg/kg/day, p.o.) in normotensive dogs, the blood pressure decreased significantly from the control value of 137±7 mmHg to 111±6 mmHg (P<0.05) at 4 weeks and to 100±4 mmHg (P<0.05) at 13 weeks (Fig. 3A). Pressor response to Ang I was markedly inhibited at 13 weeks (Fig. 3B), while pressor response (9.2±1.9 mmHg) to Ang II in a dose of 0.02 µg/kg (i.v.) was not affected (data was not shown). ACE activities of the aorta and kidney after 13 weeks administration of SA446 were also inhibited by 88 and 95%, respectively, compared with control values (Table 1).

Changes in plasma ACE activity, PRA, plasma Ang I concentration and PAC after long-term administration of different doses of SA446 (10, 30 and 100 mg/kg/day, p.o.) are summarized in Table 2 and Fig. 4. SA446 produced a dose-dependent inhibition of plasma ACE activity at 4 and 13 weeks after the administration, although there was no significant difference between the inhibitions at 4 and 13 weeks. Captopril (100 mg/kg/day, p.o.) was also found to inhibit plasma ACE activity by 52.4% at 13 weeks after the administration, the inhibition being significantly less than that of 88.2% by SA446 (100 mg/kg/day, p.o.). SA446 significantly increased PRA at 4 and 13 weeks in a dose-dependent fashion. Plasma Ang I concentration also increased dose-dependently. In contrast, PAC was significantly reduced by a long-term administration of SA446 (100 mg/kg/day, p.o.).

4. Antihypertensive effect in 2-kidney, 1-clip renal hypertensive dogs: Figure 5 shows changes in mean blood pressure, plasma ACE activity, PRA and PAC after oral administration of SA446 (5 mg/kg) in two dogs, in which mean blood pressures were elevated from 71 and 66 mmHg to 107 and 116 mmHg, respectively, 4 weeks after clipping the left kidney.

| Compounds | Dose (mg/kg) | ACE activity (mU/mg protein) |
|-----------|-------------|-----------------------------|
|           |             | Aorta          | Kidney         |
| Control   | 0           | 0.25±0.01      | 11.1±2.55     |
| SA446     | 100         | 0.03±0.01      | 0.57±0.15     |

Each value represents the mean±S.E. of 5 dogs.
Table 2. Changes in plasma ACE activity following the long-term administration of SA446 in normotensive dogs

| Dose (mg/kg/day, p.o.) | Inhibition % of ACE1 |
|-----------------------|-------------------|
|                       | 4 weeks | 13 weeks |
| SA446 10              | 61.4±5.8 (5)      | 64.9±4.9 (9) |
| 30                    | 79.0±4.8 (7)      | 70.2±5.8 (10) |
| 100                   | 87.7±1.9 (12)     | 88.2±1.8 (15) |
| Captopril 100         |         | 52.4±7.5* (3)   |

1 Each value represents the mean±S.E. ACE activities before the administration of drugs were taken as 100%. Figures in parentheses show the number of experiments. *Significantly different from the 100 mg/kg SA446 administration group, P<0.05.

Fig. 4. Changes in plasma renin activity (PRA), plasma angiotensin I concentration (P Angl) and plasma aldosterone concentration (PAC) during 13 weeks (W) administration of SA446 (10, 30 and 100 mg/kg/day, p.o.) in normotensive dogs. Each column represents the mean±S.E. of the number of dogs in parentheses. Significantly different from the initial value (before), aP<0.05, bP<0.01.
renal artery. The hypotension was caused 1, 3 and 6 hr after the application of SA446 and tended to be reversed 24 hr later. Plasma ACE activity was almost completely inhibited 1, 3 and 6 hr later and was suppressed approximately 50% at 24 hr. The maximum increase of PRA and decrease of PAC were observed 1 or 3 hr later. Figure 6 summarizes the maximum hypotensive effect of SA446 (5 mg/kg) in renal hypertensive dogs 2 or 4 weeks after clipping the left renal artery (N=4 or 5). Mean blood pressure was elevated from a pre-clipping value of 94.8 mmHg to 144.5 mmHg at 2 weeks and to
123.2 mmHg at 4 weeks. PRA was raised in all cases from a pre-clipping level of 2.4 ng Ang I/ml/hr to 9.0 ng Ang I/ml/hr at 2 weeks and to 9.8 ng Ang I/ml/hr 4 weeks after clipping. Maximum decrease of blood pressure produced by SA446 was 37.5±5.7 and 23.8±1.6 mmHg 2 and 4 weeks after clipping, respectively, which were significantly greater than the value prior to clipping (10.8±2.7 mmHg). The maximum effect of captopril (5 mg/kg) was 15.0±2.5 mmHg (N=3) 4 weeks after clipping (data was not shown in the figure), which was significantly (P<0.05) less than that of SA446.

Discussion

Oral administration of SA446 decreased blood pressure in normotensive dogs; and during repeated administration of SA446 for 13 weeks, the hypotension was maintained. The depressor response to SA446 was greater in 2-kidney, 1-clip renal hypertensive dogs than in normotensives. SA446 is reportedly a potent ACE inhibitor, which has an IC50 of 6 nM toward semi-purified rabbit lung ACE (1). In the present study, SA446 inhibited plasma ACE activity and the pressor response to Ang I. In addition, it provoked an elevation of PRA and plasma Ang I concentration, and a reduction of PAC which are supposed to be associated with the ACE inhibition and with a subsequent reduction of circulating Ang II and aldosterone secretion. Increase in plasma Ang I concentration and decrease in plasma Ang II concentration may result in a rise of PRA via the negative feedback mechanism. Therefore, the hypotensive effect of SA446 is most probably due to the ACE inhibition and the consequent prevention of Ang II generation. In fact, SA446 showed a remarkable hypotensive effect in 2-kidney, 1-clip renal hypertensive dogs, in which the hypotension is considered to be dependent on activated RAS.

SA446 inhibited tissue ACE activities of the aorta and kidney. Despite a possible reactivation from SA446-induced inhibition during in vitro procedures, such as tissue homogenization, incubation in media, etc. (13–15), tissue ACE activities were reduced by approximately 90%, suggesting an intense inhibition of tissue ACE by SA446. Ang I is rapidly converted to Ang II in isolated blood vessels (16). Ang II produces a vasoconstriction, possibly due to a direct action on vascular smooth muscles (17) and to an action on adrenergic nerve terminals, resulting in increased release of transmitter noradrenaline (18). The contractile response of arteries induced by the nerve stimulation is potentiated by Ang II in concentrations insufficient to induce the direct vasoconstriction, and the potentiating effect on adrenergic nerves is enhanced in arteries from chronic renal hypertensive dogs (19). These findings suggest that vascular ACE plays an important role in the maintenance of blood pressure; thereby, the reduction by ACE inhibitors of Ang II production in the vascular wall is expected to be a mechanism underlying a decrease of blood pressure.

The ACE inhibitory activity of SA446 in vitro is about 5 times as potent as that of captopril, and the activity of SA446 to inhibit the pressor response to Ang I in anesthetized dogs is about 3 times higher than that of captopril (1). The present study clearly demonstrated in conscious dogs that SA446 had more potent hypotensive effect and longer inhibitory effect on the pressor response to Ang I than captopril. Tolerance to SA446 does not appear to develop in dogs, because the decrease in blood pressure and the inhibition of plasma ACE activity were sustained during the oral administration of SA446 for 13 weeks. Kokubu et al. (20) have reported that long-term blockade of ACE by captopril activates plasma ACE in normotensive rats. However, plasma ACE activity was not increased in dogs treated with SA446 for 13 weeks.

Inhibition of kinin degradation may participate in the hypotensive effect of SA446, as ACE is identical with kininase II (21). Measurements of plasma bradykinin by radioimmunoassay after acute or subacute administration of ACE inhibitors have provided conflicting results in humans, because of difficulties in its measurement (22–25). Whether or not the bradykinin potentiating effect participates in the hypotensive mechanism remains to be clarified.
In conclusion, SA446 significantly decreased blood pressure in 2-kidney, 1-clip renal hypertensive and normotensive conscious dogs with the suppression of ACE activity in plasma, aortae and kidneys. The hypotensive effect of SA446 was more potent and persistent than that of captopril. The hypotension may be associated mainly with an inhibition of ACE in plasma and also in the vascular wall.

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References

1 Iso, T., Yamauchi, H., Suda, H., Nakata, K., Nishimura, K. and Iwao, J.: A new potent inhibitor of converting enzyme: (2R, 4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA446). Japan. J. Pharmacol. 31, 875–882 (1981)

2 Nakata, K., Iwatani, T., Horiuchi, M., Kito, H., Yamauchi, H. and Iso, T.: Species difference of (2R, 4R)-2-[(o-hydroxyphenyl)-3-(3-mercapto-propionyl)-4-thiazolidinecarboxylic acid (SA446) in inhibition of angiotensin converting enzyme. Japan. J. Pharmacol. 40, 367–372 (1986)

3 Ondetti, M.A., Rubin, B. and Cushman, D.W.: Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. Science 196, 441–444 (1977)

4 Funae, Y., Komori, T., Sasaki, D. and Yamamoto, K.: Inhibition of angiotensin I converting enzyme: (4R)-3-[(2S)-3-mercaptop-2-methylpropanoyl]-4-thiazolidinecarboxylic acid (YS-980). Biochem. Pharmacol. 29, 1543–1547 (1980)

5 Sweet, C.S., Gross, D.M., Arbegast, P.T., Gaul, S.L., Britt, P.M., Ludden, C.T., Woitz, D. and Stone, C.A.: Antihypertensive activity of N-[(S)-1-ethoxycarbonyl]-3-phenylpropionyl]-L-Ala-L-Pro (MK-421), an orally active converting enzyme inhibitor. J. Pharmacol. Exp. Ther. 216, 558–566 (1981)

6 Schölkens, B.A., Becker, H.A. and Kaiser, J.: Cardiovascular and antihypertensive activities of the novel non-sulfhydryl converting enzyme inhibitor 2-[N-[(S)-1-ethoxycarbonyl]-3-phenylpropionyl]-L-alanyl]-(1S, 3S, 5S)-2-azabiclyclo [3. 3. 0] octane-3-carboxylic acid (Hoe 498). Arzneimittel-Forsch. 34, 1417–1425 (1984)

7 Muirhead, E.F., Previtt, R.L., Brooks, B. and Brosius, W.L.: Antihypertensive action of the orally active converting enzyme inhibitor (SQ 14225) in spontaneously hypertensive rats. Circ. Res. 43, 53–59 (1978)

8 Fyhrquist, F., Forslund, T., Tikkanen, I. and Gronhagen-Riska, C.: Induction of angiotensin I-converting enzyme in rats lung with captopril (SO 14225). Eur. J. Pharmacol. 67, 473–475 (1980)

9 Unger, T., Schull, B., Hubner, D., Yukimura, T., Lang, R.E., Racher, W. and Ganten, D.: Plasma-converting enzyme activity does not reflect effectiveness of oral treatment with captopril. Eur. J. Pharmacol. 72, 255–259 (1980)

10 Waer, B., Brunner, H.R., Brunner, D.B., Curtet, A.L., Turini, G.A. and Gavras, H.: Discrepancy between antihypertensive effect and angiotensin converting enzyme inhibition by captopril. Hypertension 2, 236–242 (1980)

11 Miyazaki, M., Okunishi, H., Nishimura, K. and Toda, N.: Vascular angiotensin-converting enzyme activity in man and other species. Clin. Sci. 66, 39–45 (1984)

12 Horiuchi, M., Fujimura, K., Terashima, T. and Iso, T.: Method for determination of angiotensin-converting enzyme activity in blood and tissue by high-performance liquid chromatography. J. Chromatogr. 233, 123–130 (1982)

13 Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M. and Skeggs, L.T.: Hydrolysis of bradykinin by angiotensin converting enzyme. Circ. Res. 34, 824–827 (1974)

14 Williams, G.H. and Hollegen, N.K.: Accentuated vascular and endocrine response to SO14,225 in hypertension. N. Engl. J. Med. 297, 184–188 (1977)

15 Swartz, S.L., Williams, G.H., Hollegen, N.K., Moore, T.J. and Dluhy, R.G.: Converting enzyme inhibition in essential hypertension: The hypertensive response does not reflect only reduction of angiotensin II formation. Hypertension 1, 106–111 (1979)

16 Okunishi, H., Miyazaki, M. and Toda, N.: Evidence for a putatively new angiotensin II-generating enzyme in the vascular wall. J. Hypertension 2, 277–284 (1984)

17 Toda, N., Hayashi, S. and Miyazaki, M.: Contractile responses of isolated dog mesenteric arteries to angiotensin I, II and III. Japan. J. Pharmacol. 28, 527–534 (1978)

18 Hughes, J. and Roth, R.H.: Evidence that angiotensin enhances transmitter release during sympathetic nerve stimulation. Br. J. Pharmacol. 41, 239–255 (1971)

19 Toda, N., Miyazaki, M. and Okamura, T.: Vascular neuroeffector function in two-kidney, one-clip hypertensive dogs. J. Hypertension 3,
20 Kokubu, T., Ueda, E., Ono, M., Kawabe, T., Hayashi, Y. and Kan, T.: Effects of captopril (SQ14,225) on the renin-angiotensin-aldosterone system in normal rats. Eur. J. Pharmacol. 62, 269–275 (1980)

21 Roulston, J.E., MacGregor, G.A. and Bird, R.: The measurement of angiotensin-converting enzyme in subjects receiving captopril. N. Engl. J. Med. 303, 397 (1980)

22 Dux, S., Rosenfeld, J., Yaron, A. and Carmel, A.: More on captopril and measurement of angiotensin-converting enzyme. N. Engl. J. Med. 304, 423–424 (1981)

23 Cohen, M.L. and Kurz, K.: Captopril and MK-421: stability on storage, distribution to the central nervous system, and onset of activity. Fed. Proc. 42, 171–175 (1983)

24 Caldwell, P.R.B., Seegal, B.C., Hsu, K.C., Das, M. and Soffer, R.L.: Angiotensin-converting enzyme: vascular endothelial localization. Science 191, 1050–1051 (1976)

25 Ryan, J.W., Ryan, U.S., Schults, D.R., Whitaker, C. and Chung, A.: Subcellular localization of pulmonary angiotensin-converting enzyme (kininase II). Biochem. J. 146, 497–499 (1975)