A NEW POTENT INHIBITOR OF CONVERTING ENZYME: 
(2R,4R)-2-(2-HYDROXYPHENYL)-3-(3-MERCAPTOPROPIONYL)-
4-THIAZOLIDINECARBOXYLIC ACID(SA446)

Tadashi ISO, Hideyasu YAMAUCHI, Hiroshi SUDA, Katsuhiko NAKATA, 
Kazuo NISHIMURA and Jun-ichi IWAO
Research Laboratory, Santen Pharmaceutical Co., Ltd., 3-9-19 Shimoshinjo-cho, 
Higashiyodogawa-ku, Osaka 533, Japan
Accepted June 10, 1981

Abstract—(2R, 4R)-2-(2-Hydroxyphenyl)-3-(3-mercaptopropionyl)-4-
thiazolidinecarboxylic acid (SA446) is a novel potent converting enzyme 
inhibitor having a sulfhydryl group in the molecule. SA446 inhibited the 
activity of semi-purified rabbit lung converting enzyme (IC50=6 nM). The 
contractile response of isolated guinea pig ileum to angiotensin I (AI) was 
markedly inhibited by SA446 (IC50=28 nM). On the other hand, SA446 
augmented the contraction to bradykinin (BK) (AC50=0.7 nM), but did 
not affect the contraction caused by angiotensin II (AII), acetylcholine and 
histamine. These in vitro potencies of SA446 were 4 to 5 times larger 
than those of captopril. SA446 inhibited the pressor response to AI in rats 
(ID50=0.06 mg/kg, i.v., 0.48 mg/kg, p.o.) and dogs (ID50=0.01 mg/kg, 
i.v.). SA446 augmented the depressor response to BK (AD50=0.009 mg/
kg, i.v.), but did not affect the pressor responses to AII and norepinephrine 
in rats. These in vivo activities of SA446 in dogs were more potent than 
those of captopril, but the reverse was seen in rats. Oral administration of 
SA446 had a hypotensive effect on two-kidney, one-clip renal hypertensive 
rats and spontaneously hypertensive rats, at doses over 3 and 10 mg/kg, 
respectively. However, the blood pressure of normotensive and DCCA-salt 
hypertensive rats was not affected by SA446, in doses up to 100 mg/kg. 
These results indicate that oral SA446 is a potent active inhibitor of converting 
enzyme and may be classed as an antihypertensive agent.

Orally active inhibitors of converting enzyme such as captopril (2, 3), YS980, (4, 5) 
and MK-421 (6) have received a great deal of attention as antihypertensive therapy for 
patients with renovascular and essential hypertension. Recently, a compound with 
more potent activity than YS980, (2R, 4R)-
2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA-
A preliminary study on SA446 was reported at the 
53rd General Meeting of the Japanese Pharmacolo-
Fig. 1. Chemical structure of SA446.

Japan. J. Pharmacol. 31, 875–882 (1981)
(ACE) inhibitor and its effects on blood pressure in experimental hypertensive rats are reported herein.

MATERIALS AND METHODS

In vitro studies: The activity of converting enzyme inhibitors was assayed using semi-purified enzyme and isolated guinea pig ileum. Converting enzyme was partially purified from rabbit lung, according to the method of Das and Soffer (7) and its specific activity was 22.5 units/mg protein. The enzyme activity was measured, according to the method of Cushman and Cheung (8) as follows: 5 mU of semi-purified enzyme was incubated with 5 mM Hip-His-Leu as substrate in a total volume of 0.25 ml for 10 min at 37°C in 0.1 M potassium phosphate buffer (pH 8.3) containing 0.3 M NaCl. The reaction was terminated by addition of 0.75 ml of 3% metaphosphoric acid and hippuric acid liberated from the substrate was determined using reversed phase high performance liquid chromatography.

Male Hartley strain guinea pigs weighing 350 to 500 g were sacrificed by a blow on the head and segments of the ileum were dissected. The tissues were suspended in 20 ml organ bath filled with Tyrode solution at 30°C and bubbled with 95% O₂ + 5% CO₂. Contractile responses to agonists were measured isometrically with a force displacement transducer (Nihon Kohden Kogyo, Co., Ltd., SB-1T-H) for 90 sec at 10 min intervals in the presence of 0.5 g load. The compounds tested were added to the bath 5 min before addition of agonists. Dose-response curves for agonists were obtained by the single dose technique, and the contractile activity was represented as percent contraction relative to the maximal response to acetylcholine (10⁻⁵ M).

Pressor and depressor response to angiotensin I, bradykinin and other agonists: Male Wistar rats weighing 200 to 230 g and adult mongrel dogs of either sex weighing 10 to 16 kg were used. The right femoral arteries of urethane anesthetized rats and pentobarbital anesthetized dogs were cannulated, and the blood pressure was monitored with a pressure transducer apparatus (Nihon Kohden Kogyo, MPU-0.5A). Drugs were injected into the right femoral vein through a cannula, and pressor and depressor responses to angiotensin I, angiotensin II, norepinephrine and bradykinin were measured before and after administration of SA446.

The effects of orally administered SA446 were also examined in conscious animals. The cannulas were implanted into the right femoral artery and vein of rats under ether anesthesia, and also implanted into the right common carotid artery through the superior thyroid branch and carotid vein of dogs under pentobarbital anesthesia (9). The exterior ends of both cannulas were passed under the skin to emerge at the dorsal side of the neck. After the animals recovered from the anesthesia and trauma of surgery, the pressor response to angiotensin I was measured before and after oral administration of SA446.

Hypotensive effects on experimental hypertensive rats: Two-kidney, one-clip renal hypertensive rats were prepared in male Wistar animals weighing 170 to 190 g, by placing silver clips (0.2 mm internal diameter) on the left renal artery, with the right kidney being left intact. Six weeks later, the animals were used in the experiments. DOCA-salt hypertension was produced in male Wistar rats weighing 170 to 190 g by left nephrectomy and administration of DOCA-salt. The administration regimen consisted of subcutaneous injection of 15 mg/kg of DOCA weekly for 10 weeks and these rats were fed a 3% NaCl diet, ad libitum. The animals were used in the experiments about 10 weeks after operation. Male spontaneously hypertensive rats were purchased from
Charles River Breeding Farm (Charles River, New York) and used at 300 to 400 g of weight (40–43 weeks of age). As normotensive rats, male Wistar rats weighing 200 to 300 g (7–8 weeks of age) were used. The blood pressure of these animals was measured through cannula implanted into the femoral artery in conscious rats before and after oral administration of SA446.

Drugs: SA446 was synthesized at Santen Pharmaceutical Co., Ltd. Captopril was also synthesized in our laboratory and its physical constants were as follows: mp 104–106°C, [α]D25 =-131.0° (c=2.0, ethyl alcohol). These test compounds were dissolved in 0.9% NaCl solution and adjusted to pH 7.0 with NaOH solution. In conscious animals, the compounds suspended in 0.5% tragacanth and put into gelatin capsules were given orally to rats and dogs, respectively. Hip-His-Leu was purchased from Protein Research Foundation and dissolved in 100 mM NaCl. Angiotensin I (AI), angiotensin II (AII) and bradykinin (BK) were dissolved in 0.9% NaCl. Acetylcholine chloride, histamine dihydrochloride and L-norepinephrine bitartrate were dissolved in 0.9% NaCl and adjusted to pH 7.0 with NaOH solution. Deoxycorticosterone acetate (DOCA) was suspended in 0.9% NaCl solution containing Tween 40.

RESULTS

Figure 2 shows the effects of SA446 on the converting enzyme activity and the contractile responses of guinea pig ileum to AI and BK in vitro. The compound inhibited the enzyme activity and the contractile response to AI, concentration dependently. On the other hand, the contractile response to BK was augmented by the lower concentration of SA446. The IC50 values (concentration producing 50% inhibition) and AC50 values (concentration producing 50% augmentation), determined by linear regression of the dose-response curves are shown in Table 1.

As illustrated in Fig. 3, the dose-response relationships of AI and BK were shifted to the right and left by SA446, respectively, but there was no effect on the contractile responses to AII, acetylcholine and histamine.

\[\text{Inhibition of converting enzyme and AI}\]

\[\text{Augmentation of BK}\]

\[\text{Concentration of SA446 (-Log M)}\]

Fig. 2. Effect of SA446 on the activity of converting enzyme semi-purified from rabbit lung (– – – – ) and contractile response of isolated guinea pig ileum to \(10^{-7}\) g/ml of angiotensin \(\text{I (AI)} (– \text{- - - -})\) and \(5 \times 10^{-9}\) g/ml of bradykinin (BK) (– – – – – ). Each point represents the means±S.E. of at least 4 determinations.
at concentrations up to $10^{-4}$ M (data not shown).

Figure 4 shows the inhibitory effects of SA446 on the pressor response to Al in rats and dogs. The effects of intravenously administered SA446 lasted for about 1 hr in anesthetized rats and dogs, at a dose of 0.1 mg/kg and 0.05 mg/kg, respectively. On the other hand, orally administered SA446 inhibited the Al-induced pressor response.

**Fig. 3.** Concentration-effect curves for contractile responses of isolated guinea pig ileum to (a) angiotensin I (Al) and (b) bradykinin (BK) in the absence (●) and presence of $3 \times 10^{-9}$ M (△), $10^{-8}$ M (○) and $3 \times 10^{-8}$ M (▽) of SA446. SA446 was added to the bath 5 min before addition of the agonists. The contractile activities were expressed as percent contraction relative to the maximal response to acetylcholine. Each point represents the means±S.E. of at least 4 determinations.

**Fig. 4.** Effect of (a) intravenously (0.1 mg/kg to rats, 0.05 mg/kg to dogs) and (b) orally (1 mg/kg) administered SA446 on pressor response to Al in rats (●—●) and dogs (○—○). Al was given i.v. in a dose of 0.3 μg/kg in rats and anesthetized dogs, and 0.1 μg/kg in conscious dogs. Each point represents the means±S.E. of 5 animals.
for about 3 hr in conscious rats and for more than 7 hr in conscious dogs, at a dose of 1 mg/kg. The inhibitory effects of SA446 administered intravenously and orally to dogs were more prominent and persistent than the effects on rats.

As shown in Fig. 5, the dose-response curve of the pressor response to Al was shifted to the right by intravenous administration of SA446 (0.05 mg/kg). However, the pressor responses to All and norepinephrine were not affected by the same dose of SA446 given to anesthetized rats. On the other hand, the BK-induced depressor response in anesthetized rats was significantly augmented by a smaller dose of SA446 (0.01 mg/kg, i.v.) as shown in Fig. 6.

The in vitro and in vivo activities of captopril were also determined in the same assay systems and the activities were compared with those of SA446 (Table 1). The potency ratio of SA446 to captopril was 4 to 5 in the in vitro assay systems. In the in vivo assay systems, the potency ratio of SA446 to captopril varied considerably according to the animal species, and the ratio in the inhibitory effect on the pressor response to Al in rats was 0.5 (i.v.) and 0.35 (p.o.), whereas that in dogs was 3 (i.v.). Although the inhibitory effect of SA446 on the pressor response to Al in rats was less potent than that of captopril, the augmentation effect of the inhibitors on the depressor response to BK was almost equipotent in this species.

The hypotensive effects of SA446 on various hypertensive rats are shown in Fig. 7. SA446 produced marked hypotensive effects on renal hypertensive rats at doses of over 3 mg/kg (p.o.). Blood pressure was also decreased by SA446 at doses of over 10 mg/kg in spontaneously hypertensive rats, but the effect was moderate compared with that on renal hypertensive rats. On the other hand, the compound did not show any
significant effect on blood pressure in normotensive and DOCA-salt hypertensive rats, at doses up to 100 mg/kg.

**DISCUSSION**

Converting enzyme converts the biologically inactive Al to the potent vasopressor form All and also degrades bradykinin, a depressor peptide (10, 11). In the present studies, SA446, a new sulfhydryl compound, was shown to produce potent inhibitory effects on converting enzyme semi-purified from rabbit lung. Also, the compound inhibited the contractile response of isolated guinea pig ileum to Al and augmented the response to BK, whereas it did not affect the responses to the other agonists. These in vitro effects of SA446 were 4 to 5 times as potent as those of captopril. In the in vivo assay systems, it was also demonstrated

| Compound  | Converting enzyme (IC50 nM) | Guinea pig ileum (IC50 nM) | Pressor response to Al (ID50 mg/kg) | Depressor response to BK (AD50 mg/kg) |
|-----------|-----------------------------|---------------------------|-----------------------------------|--------------------------------------|
|           |                             | Al                        | Rat                               | Dog                                  |
| SA446     | 6                           | 28                        | 0.063                             | 0.008                                |
| Captopril | 30                          | 130                       | 0.030                             | 0.010                                |

Values were calculated from the least fit linear regression lines of dose-response curves obtained in at least from 4 experiments. IC50 and AC50 were concentrations of compounds producing 50% inhibition and augmentation, respectively. ID50 and AD50 were doses producing 50% inhibition and augmentation, respectively.

Fig. 7. Hypotensive effect of SA446 in (a) normotensive rats, (b) two-kidney, one-clip renal hypertensive rats, (c) spontaneously hypertensive rats and (d) DOCA-salt hypertensive rats. Initial mean blood pressure of these rats was 110±2, 211±2, 183±3 and 178±3 mmHg (means±S.E.), respectively. SA446 was given p.o. in a dose of 3 mg/kg (---O---), 10 mg/kg (---V---), 30 mg/kg (---\(\nabla\)---) or 100 mg/kg (---□---), and control rats were given 0.5% tragacanth (---●---). Each point represents the means±S.E. of 5 to 8 animals.
that SA446 has a potent inhibitory effect on converting enzyme. However, the potency of SA446 varied according to the animal species, while the species difference was not observed in the case of captopril. The potencies of SA446 were higher in dogs than in rats, and the ratio was about 6. However, the potency of captopril was almost equal in dogs and rats. The species difference observed in the present studies is a most interesting finding. In this respect, we are now investigating the blood level, distribution, excretion, metabolism and protein binding etc., after administration of SA446.

It is generally assumed that the inhibition of renin-angiotensin system is the principal mechanism of converting enzyme inhibitors involved in the production of hypotensive effects. In fact, SA446 exhibited remarkable hypotensive effects on two-kidney, one-clip renal hypertensive rats, an experimental model of renin dependent hypertension (12, 13), and had no effects on the blood pressure in DOCA-salt hypertensive rats, a low renin model of hypertension (14). However, the compound also reduced blood pressure in spontaneously hypertensive rats, though higher doses were required. Since plasma renin activity of adult spontaneously hypertensive rats shows no difference or is lower than levels in normotensive adult rats (15), mechanisms other than renin-angiotensin system may be involved in the hypotensive effects of SA446. It has been reported that kininase II is identical with converting enzyme and the affinity of BK for the enzyme is greater than that of AI (10). Furthermore, a significant part of the acute antihypertensive effect of captopril may be mediated by kinins (16). Thus it is considered that not only the renin-angiotensin system but also the kallikrein-kinin system may play important roles in the development of hypotensive effects of SA446. This possibility might be supported by the present finding that SA446 produced more prominent effects on the responses to BK than those to AI.

Acknowledgement: We thank Prof. K. Yamamoto, Department of Pharmacology, Osaka City University Medical School, for helpful advice.

REFERENCES
1) Iso, T., Yamauchi, H., Suda, H., Nakajima, N., Nishimura, K., Takada T., Horiuchi, M., Nakata, K. and Iwao, J.: Pharmacological studies on SA446, a new angiotensin I-converting enzyme inhibitor. Japan. J. Pharmacol. 30, Supp. 136P (1980)
2) 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)
3) Rubin, B. and Antonaccio, M.J.: Pharmacology of antihypertensive drugs. Edited by Scriabine, A., p. 21-42, Raven Press, New York (1980)
4) Mita, I., Iwao, J., Oya, M., Chiba, T. and Iso, T.: New sulfhydryl compounds with potent antihypertensive activities. Chem. Pharm. Bull. 26, 1333-1335 (1978)
5) Funae, Y., Komori, T., Sasaki, D. and Yamamoto, K.: Inhibitor of angiotensin I converting enzyme: (4R)-3-[(2R)-3-mercapto-2-methylpro-pionyl]-4-thiazolidine-carboxylic acid (YS-980). Biochem. Pharmacol. 29, 1543-1547 (1980)
6) Patchett, A.A., Harris, E., Tristram, E.W., Wyvrett, M.J., Wu, M.T., Taub, D., Peterson, E.R., Ikeler, T.J., ten Broeke, J., Payne, L.G., Ondeyka, D.L., Thorsett, E.D., Greenlee, W.J., Lohr, N.S., Hoffmmer, R.D., Joshua, H., Rytle, W.V., Rothrock, J.W., Aster, S.D., Maycock, A.L., Robinson, F.M. and Hirschmann, R.: A new class of angiotensin-converting enzyme inhibitor. Nature 288, 280-283 (1980)
7) Das, M. and Soffer, R.L.: Pulmonary angiotensin-converting enzyme: structural and catalytic properties. J. biol. Chem. 250, 6762-6768 (1975)
8) Cushman, D.W. and Cheung, H.S.: Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20, 1637-1648 (1971)
9) Saxena, P.R.: A simple technique for the recording of arterial blood pressure in unanesthetized dogs. Life Sci. 9, 129-135 (1970)
10) Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M. and Skeggs, L.T.: Hydrolysis of bradykinin by angiotensin converting enzyme. Circulation Res. 34, 824–827 (1974)

11) Erdos, E.G.: Conversion of angiotensin I to angiotensin II. Am. J. Med. 60, 749–759 (1976)

12) Brunner, H.R., Kirshman, J.D., Sealey, J.E. and Laragh, J.H.: Hypertension of renal origin: Evidence of two different mechanisms. Science 174, 1344–1346 (1971)

13) Swales, J.D., Thurston, H., Queiroz, F.P., Medina, A. and Holland, J.: Dual mechanism for experimental hypertension. Lancet 2, 1181–1184 (1971)

14) Pettinger, W.A., Marchelle, M. and Augusto, L.: Renin suppression by DOC and NaCl in the rat. Am. J. Physiol. 221, 1071–1074 (1971)

15) Kawashima, K., Shiono, K. and Sokabe, H.: Variation of plasma and kidney renin activities among substrains of spontaneously hypertensive rats. Clin. exp. Hypertension 2, 229–245 (1980)

16) Carretero, O.A., Miyazaki, S. and Scicle, A.G.: Role of kinins in the acute antihypertensive effect of the converting enzyme inhibitor, captopril. Hypertension 3, 18–22 (1981)