Pharmacological Profiles of CS-622, a Novel Angiotensin Converting Enzyme Inhibitor

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Abstract—CS-622 is a prodrug type ACE inhibitor with a thiazepin ring. Its active form, CS-622 diacid, was slightly more potent than enalaprilat in inhibiting ACE isolated from rabbit lung. The inhibitory potency of CS-622 diacid on isolated rat aorta was 3 times that of enalaprilat. The inhibitory action of enalaprilat was abolished quickly by washing the aortic strip with drug-free solution, whereas that of CS-622 diacid was abolished only slowly. This difference suggests that CS-622 diacid binds to vascular ACE more firmly than enalaprilat. By oral administration, CS-622 was 3 times more potent than enalapril, and its onset of action was faster than that of enalapril, suggesting that the conversion of CS-622 to its active diacid occurs faster than the conversion of enalapril. Although CS-622 diacid was only slightly more potent than enalaprilat by intravenous administration, it had a longer duration than enalaprilat. Elimination of renal excretory function potentiated the action of captopril but not that of CS-622, suggesting that unlike captopril, only a small portion of CS-622 is excreted through the kidney.

Since the development of captopril, the first orally active inhibitor of angiotensin converting enzyme (ACE) (1), many ACE inhibitors have been synthesized in an attempt to obtain more potent and long-lasting drugs. They are mostly prodrug type inhibitors (2–4), and among these agents, only enalapril is clinically used at present. Although the duration of action is sufficiently long so that the drug can be given by a once-daily regimen, enalapril is not suitable for the treatment of an emergency situation as its onset is slow (5). Besides, captopril and enalapril cause untoward effects more frequently in patients with renal failure, because the blood concentration of these agents vary with renal excretory function (6–8).

Aiming for a potent and long-lasting ACE inhibitor with rapid onset, we synthesized a series of perhydrothiazepine derivatives and assayed them for ACE inhibitory action with special attention to the onset of action and the route of excretion in vivo (9). Among these thiazepin compounds, CS-622 was found to be the most potent. X-ray analysis revealed that the conformation of the major functional groups in CS-622 molecule were restricted so that these groups bind well to hypothetical binding sites of the ACE molecule (9). We describe here in vitro and in vivo profiles of ACE inhibition produced by

\[ \text{Fig. 1. Chemical structures of CS-622 diacid and CS-622.} \]
\[ \text{H} \quad \text{COOR} \]
\[ \text{CH}_2 \text{COOH} \]
\[ \text{R} = \text{H} : \text{CS-622 diacid} \]
\[ \text{R} = \text{CH}_2 \text{CH}_3 : \text{CS-622} \]

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CS-622, whose chemical structure differs from that of existing ACE inhibitors such as enalapril (2), ramipril (3) and cilazapril (4) (Fig. 1). Since CS-622 is a prodrug type ACE inhibitor, its diacid (Fig. 1) was used for in vitro experiments and in vivo experiments with intravenous administrations.

Materials and Methods

In vitro experiments

a) Inhibition of rabbit lung ACE: The ACE used was solubilized from the particulate fraction of the rabbit lung with Nonidet-P40 and fractionated with DEAE-cellulose (DE-52, Whatman) according to the method of Das and Soffer (10).

The ACE activity was measured by essentially the same method as described by Cushman and Cheung (11) using hippurylhistidyl-leucine (HHL) as substrate. The standard assay mixture contained in a total volume of 250 μl: 25 μmoles of potassium phosphate buffer (pH 8.3), 75 μmoles of sodium chloride, 1.25 μmoles of HHL and the enzyme. The reaction was initiated by adding the enzyme in a volume of 25 μl. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by adding 250 μl of 1N HCl. Hippuric acid formed was extracted from the acidified solution into 1.5 ml of ethylacetate by mixing for 20 sec. After centrifugation, a 1.0 ml aliquot of the ethylacetate layer was transferred to a test tube and evaporated to dryness in a boiling water bath. The hippuric acid was redissolved in 1.0 ml of distilled water, and its concentration was determined from the absorbance at 228 nm (Hitachi 557 spectrophotometer) after subtraction of the zero-time control absorbance. The assays were done in duplicate.

IC50 values, the concentrations of test compounds at which a 50% inhibition of the ACE activity was achieved, were determined by linear regression of % inhibition against log concentration over a 20–80% inhibition range. Data were expressed as means±S.E.M. of 3 assays.

The Lineweaver-Burk plot data for inhibition of the ACE by CS-622 diacid were calculated from the reaction velocities at 1, 1.25, 1.67, 2.5 and 5 mM HHL concentrations in the presence of 2.2, 4.5 and 8.9 nM CS-622 diacid.

b) Inhibition on rat aorta: Male Wistar-Imamichi rats (335–400 g) were stunned by a blow on the head. Segments of the thoracic aorta were removed and dissected free of fat and connective tissue in Krebs-Henseleit solution (KHS). Two helically cut strips, approximately 2 mm wide and 30 mm long, were prepared from the adjoining portion of the same aorta as described by Furchgott and Bhdarlikom (12). Aortic strips were suspended in 30 ml organ baths containing KHS maintained at 37.0±0.5°C and aerated with a 5% CO2 : 95% O2 mixture. The composition of KHS was: 119.8 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25.0 mM NaHCO3 and 11.1 mM glucose. The isometric tension of the aorta was measured with a force-displacement transducer (Nihon Kohden, TB-612T) connected to a carrier amplifier (Nihon Kohden, AP-601G). Recordings were made on a thermal pen-writing recorder (Nihon Kohden, RJG-4124).

A force of 1.0 g was applied, and the strips were allowed to equilibrate for 90 min. Aortic strips were exposed to 30 nM of angiotensin I (AI), which produced contractions approximately equal to 90% of the maximum response. After three successive contractions of equal size had been obtained, test drugs (CS-622 diacid, enalaprilat and captopril) were added to the bath. Contractile responses to AI in the presence of a test drug were compared with control responses before the drug was added. The concentration of the drug was increased in a cumulative manner by a ratio of 3 at a 10 min interval to construct a concentration-inhibition curve. The IC50 value was determined as the concentration inhibiting by 50% the response to AI (3×10⁻⁸ M).

In another series of experiments, the
control responses to Al (1×10^{-8} M) were obtained, and aortic tissues were exposed to CS-622 diacid (3×10^{-7} M) or enalaprilat (1×10^{-6} M) for 20 min. Thereafter, the tissues were washed three times with drug-free KHS. Contractile responses to Al were obtained, and aortic tissues were exposed to CS-622 diacid (3×10^{-7} M) or enalaprilat (1×10^{-6} M) for 20 min. Thereafter, the tissues were washed three times with drug-free KHS. Contractile responses to Al were obtained at 15, 45, 75 and 105 min after removal of drugs from the bathing solution.

**In vivo experiments**

a) anesthetized rats: Male Wistar-Imamichi rats weighing 320–350 g were anesthetized with Inactin, 100 mg/kg, i.p., and two polyethylene cannulae were placed: one in the left femoral artery for measuring blood pressure and the other in the left femoral vein for injecting drugs. The arterial cannula was connected to a pressure transducer (Nihon Kohden TP-200T), and mean blood pressure and heart rate were continuously recorded on a pen-writing oscilloscope (Nihon Kohden RJG-4128). After blood pressure and heart rate were stabilized, Al was administered at a dose of 0.3 μg/kg, i.v., via the venous cannula. Intravenous administration of Al was repeated until a constant pressor response was obtained, and the diacid of a test compound at a dose of 1 μg/kg, i.v., was administered. Al challenge was repeated 5 min later. The dose of the test compound was increased in a cumulative manner by a ratio of 3, and administered at a 10 min interval up to 30 μg/kg, i.v. Each dosing was followed by an Al challenge 5 min later. The pressor responses to Al after administration of the compound were divided by the control Al response to give percent inhibition of Al pressor response, and a dose-response curve was constructed.

b) conscious rats: Male Wistar-Imamichi rats weighing 310–380 g were anesthetized with sodium pentobarbital, 30 mg/kg, i.p. The animal was surgically prepared with an aortic cannula inserted via the left femoral artery and a caval cannula inserted via the left femoral vein for measuring blood pressure and injecting drugs, respectively. The other ends of the cannulae were led under the skin and exteriorized at the back of the neck. The rat was placed in an individual cage after surgery and allowed free access to tap water but not to food. On the next day, the aortic cannula was connected to a pressure transducer (Nihon Kohden TP-200T) to record blood pressure continuously. After blood pressure and heart rate were stabilized, the control pressor response to Al at 0.3 μg/kg, i.v., was obtained. A single oral dose of CS-622 or enalapril or a single intravenous dose of the diacid of either ACE inhibitor was given. Al challenge was repeated at different time intervals after administration of the test drug to obtain the time course for inhibition of Al pressor response.

In another series of experiments, renal excretory function was eliminated by ligation of bilateral renal arteries in rats. Rats with bilateral renal arteries ligation (BRAL rat) was cannulated in the femoral artery and vein as described above. On the day following surgery, the time course of Al inhibition was observed after a single oral administration of 0.1 mg/kg CS-622 or 1.0 mg/kg captopril.

**Results**

**In vitro experiments**

a) Inhibition on rabbit lung ACE: Table 1 summarizes IC50 values of various agents for inhibition of ACE isolated from rabbit lung. CS-622 diacid was slightly more potent than enalaprilat.

![Figure 2](image)

Figure 2 shows the double reciprocal plot of the HHL concentration versus reaction velocity of ACE from rabbit lung in the presence of CS-622 diacid at various concentrations. CS-622 diacid exhibited a competitive inhibition with the substrate HHL.

b) Inhibition of contractile response of the rat aorta to Al: CS-622 diacid, enalaprilat and

|           | IC50       |
|-----------|------------|
| CS-622 diacid | 3.6 ± 0.3 nM |
| enalaprilat   | 5.7 ± 0.3 nM |

The IC50 is the concentration of the drugs producing a 50% inhibition of the cleavage of Hip-His-Leu.

Table 1. Inhibitory activity of CS-622 diacid or enalaprilat on ACE isolated rabbit lung
captopril produced a concentration-dependent inhibition of the contractile response to Al (30 nM). Figure 3 shows the concentration-inhibition curves of the three drugs. IC50 values for CS-622 diacid, enalaprilat and captopril were approximately 7.6, 23 and 110 nM, respectively. CS-622 diacid was therefore about 3 times more potent than enalaprilat and about 15 times more than captopril.

Figure 4 shows the time course for recovery of the Al induced contraction after the drug had been removed from the organ bath. Since CS-622 diacid was about 3 times more potent than enalaprilat, CS-622 diacid and enalaprilat were used at concentrations of 3 x 10^{-7} M and 1 x 10^{-6} M, respectively, in the washout experiment. In the case of enalaprilat, a full contractile response to Al was restored 15 min after the aortic preparation was

Fig. 2. Lineweaver-Burk plots for the inhibition of rabbit lung angiotensin converting enzyme by CS-622 diacid.

Fig. 3. Inhibitory effects of contractile response to Al (30 nM) after adding CS-622 diacid, enalaprilat, or captopril to the bath. The aorta was exposed to drugs for 10 min. Values are means±S.E. from five rats.

Fig. 4. Time course for recovery of Al (0.1 μM) contraction after removal of drugs. CS-622 diacid and enalaprilat were used at concentrations of 0.3 μM and 1.0 μM, respectively. The tissues were exposed to CS-622 diacid or enalaprilat for 20 min and washed three times by drug free Krebs-Henseleit solution. Contractile responses to Al were obtained at 15, 45, 75 and 105 min after washout of drugs. Asterisks indicate statistically significant difference at P<0.05 from the control values by the paired t-test. Values are means±S.E. from five rats.
washed. On the other hand, the contractile response to Al was significantly smaller than the control response up to 45 min after CS-622 diacid was removed from the bath (P<0.05). These results indicate that the inhibition of aortic ACE by CS-622 diacid lasts much longer than that by enalaprilat after the aorta was washed with drug-free KHS.

In vivo experiments: CS-622 diacid or enalaprilat, at doses cumulatively increased by a ratio of 3, was intravenously administered in anesthetized rats. Both agents inhibited pressor response to Al in a dose-related manner (Fig. 5). CS-622 diacid was as potent as or slightly more potent than enalaprilat. The ED50 value for CS-622 diacid was approximately 5 μg/kg, i.v.

The time course for inhibition of Al pressor response was obtained after a single administration of CS-622 diacid or enalaprilat at a dose of 100 μg/kg, i.v., in conscious rats. As shown in Fig. 6, both agents produced a full inhibition of Al pressor response immediately after administration. The duration of the action of CS-622 diacid was twice as long as that of enalaprilat: the time required for the Al pressor response to recover by 90% was 4 hr for CS-622 diacid vs. 2 hr for enalaprilat.

Figure 7 shows time course for inhibition of Al pressor response after a single oral administration of CS-622 or enalapril. A maximum inhibition was achieved 15 to 30 min after administration of CS-622, whereas it was achieved 60 min after enalapril. The magnitude of the maximum inhibition produced by CS-622 at 0.3 mg/kg nearly equalled that produced by enalaprilat 1.0 mg/kg. CS-622 at 0.1 mg/kg produced an inhibition greater than that produced by enalapril at 0.3 mg/kg. These data demonstrate that CS-622 was more than 3 times as potent as enalapril on oral administration and that CS-622 was faster than enalapril in its onset of action.

CS-622 or enalapril at 0.1 mg/kg, i.v., was administered in conscious rats (Fig. 8). With CS-622, a maximum inhibition was achieved immediately after administration, whereas it
was achieved 15 min after enalapril. These data suggest that conversion of CS-622 into the active metabolite, the diacid, is faster than that of enalapril.

In another series of experiments captopril or CS-622 was orally administered to normal rats and rats with bilateral renal arteries ligation (BRAL rats). The inhibition of the AI pressor response produced by 1.0 mg/kg of captopril, p.o., reached a maximum at 15 min after administration and lasted for 2 hr in normal rats (Fig. 9). Both the maximum inhibition and the duration of action produced by captopril were greatly increased in BRAL rats (Fig. 9). On the other hand, there was no difference in the duration of action produced by CS-622 between normal and BRAL rats (Fig. 9).

**Discussion**

CS-622 is a prodrug type ACE inhibitor which is deesterified in vivo to the active diacid by the action of tissue esterases. The relative potency of CS-622 diacid vs. enalaprilat varied depending upon the assay system used: in the cell-free enzyme assay using ACE extracted from rabbit lung, CS-622 diacid was slightly more potent than enalaprilat, whereas CS-622 diacid was 3 times more potent than enalaprilat on the aorta preparation isolated from rats. This difference may be attributed to a species difference between the rat and rabbit.

In the washout experiments using rat aorta, ACE inhibition achieved by enalaprilat was eliminated very quickly after washing the aorta with drug-free Tyrode's solution, whereas that achieved by CS-622 diacid was eliminated only slowly. In agreement with this
observation, inhibition of the Al pressor response produced by CS-622 diacid lasted longer than that produced by enalapril after intravenous administration in conscious rats. The restricted conformation of the CS-622 molecule, which allows firm binding to the binding sites of ACE molecule, may account for the long-lasting nature of CS-622 (9).

Single oral administrations of the parent compounds to conscious rats revealed that the onset of action following CS-622 was much faster than that following enalapril. There are two possible reasons for the difference in the onset time between CS-622 and enalapril: first, the absorption of CS-622 from the gastro-intestinal tract is faster than that of enalapril; secondly, the conversion of CS-622 to the active diacid occurs at a faster rate than that of enalapril. Although the first possibility cannot be easily discarded, the second possibility was verified by other experiments in which the parent compounds were intravenously administered in conscious rats (Fig. 8). CS-622 produced a full inhibition of the pressor response to Al immediately after intravenous administration. On the other hand, enalapril produced an inhibition which developed slowly and reached a maximum 15 min after administration. These data suggest that unlike enalapril, CS-622 is quickly deesterified to its active metabolite in the body.

An oral administration of enalapril at a dose of 1.0 mg/kg produced an inhibition of Al pressor response comparable to that produced by CS-622 at 0.3 mg/kg, indicating that CS-622 is 3 times more potent than enalapril (Fig. 7). However, the inhibition produced by enalapril at 0.3 mg/kg, p.o., was much smaller than that produced by CS-622 at 0.1 mg/kg, p.o. These data suggest that blood concentration of enalaprilat remains inappropriately low when the dose of enalapril used is reduced. A possible explanation for this is as follows: the Km value for the deesterification reaction with enalapril as substrate may be so high that some of the enalapril molecules in the blood stream are not deesterified to enalaprilat, because a prodrug at concentrations lower than its Km value is resistant to enzymatic hydrolysis. Indeed after an oral administration of enalapril, the unchanged compound was detected in the blood (13), whereas no unchanged compound was found after administration of CS-622 in rats (T. Ikeda, unpublished observation).

Since captopril is excreted through the kidney, its plasma concentration sometimes elevates to an unexpectedly high level, causing untoward effects in patients with renal failure (8). This situation was reproduced in the present study. The duration of action after an oral administration of captopril was twice as long in BRAL rats as in normal rats. However, there was no difference in the duration of action after administration of CS-622 between BRAL and normal rats. These data suggest unlike other ACE inhibitors, a small portion of CS-622 is excreted through the kidney. Indeed,
only 6.3% of the total radioactivity was found in urine after an oral administration of $^{14}$C-CS-622 in rats (T. Ikeda, unpublished observation). On the other hand, urinary excretory rates after oral administration of captopril has been reported to be 66–76% (14).

In conclusion, CS-622 is a potent and long-lasting ACE inhibitor of the prodrug type. By oral administration, CS-622 was more than three times as potent as enalapril, and its onset of action was faster than that of enalapril. Although the inhibitory potency of CS-622 diacid was only slightly higher than that of enalaprilat, its duration was longer than that of enalaprilat, probably because of firm binding to the ACE molecule.

References

1 Ondetti, M.A., Rubin, B. and Cushman, D.W.: Design of specific inhibitors of angiotensin converting enzyme: a new class of orally active antihypertensive agents. Science 196, 441–444 (1977)
2 Gross, D.M., Sweet, C.S., Ulm, E.H., Backlund, E.P., Morris, A.A., Weitz, D., Bohn, D.L., Wenger, H.C., Vassil, T.C. and Stone, C.A.: Effect of N-[(S)-1-carboxy-3-phenylpropyl]-L-Ala-L-Pro and its ethyl ester (MK-421) on angiotensin converting enzyme in vitro and angiotensin I pressor response in vivo. J. Pharmacol. Exp. Ther. 216, 552–557 (1981)
3 Becker, R.H.A., Schölkens, B.A., Metzger, M. and Schulze, K.J.: Pharmacological properties of the new orally active angiotensin converting enzyme inhibitor 2-[[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-L-alanyl]-[15,35,55]-2-aza-bicyclo[3.3.0]octane-3-carboxylic acid (Hoe 498). Arzneimittelforschung 34, 1411–1416 (1984)
4 Natoff, I.L., Nixon, J.S., Francis, R.J., Klevans, L.R., Brewster, M., Budd, J., Patel, A.T., Wenger, J. and Worth, E.: Biological properties of the angiotensin-converting enzyme inhibitor Cilazapril. J. Cardiovasc. Pharmacol. 7, 569–580 (1985)
5 Brunner, H.R., Nussberger, J. and Waber, B.: The present molecules of converting enzyme inhibitors. J. Cardiovasc. Pharmacol. 7, S2–S11 (1985)
6 Duchin, K.L., Pierides, A.M., Heald, A., Singhvi, S.M. and Rommel, A.J.: Elimination kinetics of captopril in patient with renal failure. Kidney Int. 25, 942–947 (1984)
7 Giudicelli, J.F.: Clinical pharmacology of enalapril. Presse Med. 14, 2209–2211 (1985)
8 Ferguson, R.K. and Vlasses, P.H.: The ACE inhibitors: Clinical pharmacology of captopril and enalapril. Hosp. Formul. 21, 46–60 (1986)
9 Yanagisawa, H., Ishihara, S., Ando, A., Kanazaki, T., Miyamoto, S., Koike, H., Iijima, Y., Oizumi, K., Matsushita, Y. and Hata, T.: Angiotensin-converting enzyme inhibitors: Perhydro-1,4-thiazepin-5-one-derivatives. J. Med. Chem. 30, 1984–1991 (1987)
10 Das, M. and Soffer, R.L.: Pulmonary angiotensin-converting enzyme. J. Biol. Chem. 250, 6762–6768 (1975)
11 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)
12 Furchgott, R.F. and Bhadrakom, S.: Reaction of strips of rabbit aorta to epinephrine, isoproterenol, sodium nitrate and other drugs. J. Pharmacol. Exp. Ther. 108, 129–143 (1953)
13 Tocco, D.J., Deluca, F.A., Duncan, A.E.W., Vassil, T.C. and Ulm, E.H.: The physiological disposition and metabolism of enalapril maleate in laboratory animals. Drug Metab. Dispos. 10, 15–19 (1982)
14 Esumi, Y., Takaichi, M. and Kakurai, Y.: Absorption, metabolism and excretion of SQ14225 in rats. The Clinical Report 15, 52–60 (1981)