Renin Inhibitor: Relationship Between Molecular Structure and Oral Absorption

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Common problems in developing renin inhibitors are low solubility, insufficient oral absorption, and fast hepatic clearance. We focused on the molecular structure of renin inhibitors to overcome these problems. Cyclodextrins (CD) improved the low solubility of renin inhibitors, with β-CD showing the best ability to dissolve renin inhibitors. The intestinal absorption of renin inhibitors varied with both their solubility and molecular structure. Coadministration of β-CD improved the intestinal absorption of some renin inhibitors with low solubility as measured by transport into the mesenteric vein in the absorption experiment using the rat intestinal loop. Substitutions at both the N and C terminals was essential for absorption from the small intestine. A naphthyl group at the N-terminal further improved intestinal absorption. A carrier system appeared to be involved in the intestinal absorption of some renin inhibitors. N-methylation at the amide bond of thiazolylalanine suppressed the high hepatic clearance of one of the test compounds *18* which was well absorbed from the small intestine and it improved its oral bioavailability.

KEY WORDS: renin inhibitor; cyclodextrin; intestinal permeability; *in situ* intestinal loop; oral absorption; first pass effect.

INTRODUCTION

The renin-angiotensin system plays an important role in the control of blood pressure and body fluid balance. Renin selectively cleaves its substrate angiotensinogen to angiotensin I which is further converted to angiotensin II by angiotensin converting enzyme (ACE). Many ACE inhibitors are used in the treatment of hypertension. However, ACE inhibitors cause side effects such as flushing, dry cough, and angioneurotic edema, because ACE is a rather nonselective enzyme that also cleaves bradykinin, enkephalin, and other biologically active peptides. Thus, inhibitors of the highly selective enzyme renin may be advantageous in the treatment of hypertension (1–3), and renin inhibitors are being developed (4–6). Renin is an aspartyl proteinase in the renin-angiotensin cascade and is expected to act on angiotensinogen specifically. This specificity avoids side effects intrinsic to ACE inhibitors. Most renin inhibitors are derived from modification of the peptide sequence on the transition state of angiotensinogen (7–10). However, low solubility, insufficient oral absorption, and fast hepatic clearance impeded the development of renin inhibitors as a drug (11–15), and attempts have been made to improve bioavailability (16–20). In this study, a series of renin inhibitors, was tested to evaluate the relationship between solubility and intestinal absorption, with the use of solubility enhancer.

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EXPERIMENTAL SECTION

Materials

Cyclodextrins (α-, β-, γ-, and hydroxypropyl-β-cyclodextrins) were obtained from Ensuiko Sugar Refining Co., Ltd. (Yokohama, Japan). Renin inhibitors and ceftibuten were synthesized at Shionogi Research Laboratories (Osaka, Japan). Sodium heparin (1000 J.P.S. units/ml) was obtained from Shimizu Pharmaceutical (Shizuoka, Japan). Sodium pentobarbital (Somnopentyl injection) was purchased from Pitman-Moore Inc. (Mundelein, IL). Urethane was purchased from Sigma Chemical Co (St. Louis, Mo.). All other chemicals were of the highest grade available commercially.

Solubility

The renin inhibitor (10 mg) and CD (at appropriate weight ratio to renin inhibitor) were weighed in a 10-ml centrifuge tube and 2 ml water was added. The suspension was sonicated for 10 min and vortexed for 30 min at 25°C. After centrifugation at 10,000 g for 5 min at 25°C, the supernatant was diluted with N,N-dimethylformamide, which dissolved the renin inhibitor and CD, and the content of the renin inhibitor was measured by high-performance liquid chromatography (HPLC). The diluted sample was injected onto a column (4.6 × 150 mm) packed with Nucleosil $5C_{18}$ (Chemco Scientific Co., Ltd., Osaka, Japan). The eluent was a mixture of 0.1% trifluoroacetic acid solution and acetonitrile (1:1–1:2). The sample was analyzed by UV detection at the range of 220 and 230 nm with a photodiode array UV-VIS detector (SPD-M6A, Shimadzu, Kyoto, Japan).

Animal Experiments

I) Animals

Experiments were conducted using male Sprague-Dawley rats (Japan Clea Inc., Osaka, Japan) weighing 240–300 g. Animals were fasted for about 20 hrs prior to the experiment but allowed free access to water.

II) Plasma sample analysis

The plasma sample (250 μl) was deproteinized with 750 μl acetonitrile. After immediate mixing and centrifugation for 5 min at 10,000 g and 4°C by a refrigerated centrifuge (MR-15A, Tomy Seiko Co., Ltd., Tokyo, Japan), an aliquot of the clear supernatant (850 μl) was evaporated and the residue was dissolved with the eluent (150 μl) of HPLC. The HPLC conditions were the same as the solubility section.

III) Intestinal absorption—*in situ* loop method

Rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.) and placed on a warming blanket maintained at

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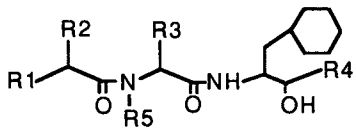
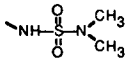
about 37°C during the experiment. The small intestine was exposed *via* a midline incision and a section of proximal jejunum, about 8 cm long and drained by a single mesenteric vein, was ligated and cannulated at the one end with a 23 gauge × 3.2 cm rounded needle (Terumo Co., Tokyo, Japan) attached to 1-ml plastic syringe containing a suspension of renin inhibitor and CD. The distal end of the loop was secured with silk suture. Sodium heparin diluted with saline (5%, v/v) was administered *via* tail vein prior to the experiment to avoid coagulation in the mesenteric vein. After 0.8 ml of the suspension of renin inhibitor (1.5 mg/ml) added with β-CD (an appropriate weight ratio to renin inhibitor) was injected directly into the lumen of the ligated intestinal loop, all mesenteric venous blood from the loop was collected continuously at 10-min intervals for 60 min from a mesenteric venous cannula (23 gauge needle attached to a silicone tube (0.51 mm i.d., 0.94 mm o.d.)). Rat blood was collected for transfusion from the abdominal aorta of other three rats in a 50-ml plastic syringe supplemented with 1 ml of 5% heparinized saline. This blood was warmed at 37°C before use and infused simultaneously *via* the tail venous cannula at approximately the same rate as that of blood drained from the mesenteric venous cannula (40 ml/hr) using an infusion pump (Model STC-523, Terumo Co., Tokyo, Japan). The intestinal loop was covered with wrapping film to keep it moist. The volume of each blood sample was determined gravimetrically based on a specific gravity of 1.0. The plasma concentration of renin inhibitor of each blood sample was determined by HPLC.

IV) Oral administration

The suspension of renin inhibitor containing β-CD was orally administered to the conscious rat with a gastric tube (renin inhibitor; 20 mg/4 ml/kg, β-CD; 140 mg/4 ml/kg). The blood sample was collected *via* the jugular vein cannulated with a polyethylene tube (0.58 mm i.d. and 0.96 mm o.d.). The plasma concentration of renin inhibitor was measured

Table I. Molecular Structure of Renin Inhibitors



Compd	R1	R2	R3	R4	R5
1					H
2					H
3					H
4					H
5					H
6					H
7					H
8					H
9					H

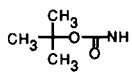
Compd	R1	R2	R3	R4	R5
10					H
11					H
12					H
13					H
14					H
15					H
16					H
17					H
18					H
CH3-18					CH_3

Table II. Solubility of Renin Inhibitors

Compound	Solubility (mg/ml, 37°C)		
	Water	JP-1[a]	JP-2[a]
3	0.20		0.20
9	0.001		
12	0.106	0.49	0.085
13	0.108	0.43	0.084
14	0.006	2.68	0.014
15	0.002	1.94	0.002
16	0.001		
CH_3-18	0.06		

[a] JP-1 is hydrochloric acid buffer, pH 1.2, and JP-2 is phosphate buffer, pH 6.8.

by HPLC. The renin inhibitor dissolved in 5% Tween 80 saline was administered intravenously *via* the tail vein to calculate the absolute bioavailability for the oral administration.

V) First pass effect

Rats were anesthetized with urethane (1.2 g/kg i.p.) and placed on a warmed blanket maintained at about 37°C for the experiment. After a midline incision, about 500 μl of renin inhibitor solution (2 mg/2 ml/kg, 5% Tween 80 saline) was infused for 15 min into the caudal or the portal vein cannulated with a 23 gauge needle attached to a silicone tube (0.51 mm i.d., 0.94 mm o.d.) using a 5-ml plastic syringe connected with an infusion pump (Model STC-531, Terumo Co., Tokyo, Japan). The blood sample was collected *via* the jugular vein cannulated with a polyethylene tube (0.58 mm i.d. and 0.96 mm o.d.). The plasma concentration of renin inhibitor was measured by HPLC.

RESULTS AND DISCUSSION

Solubility of Renin Inhibitor

Some renin inhibitors in Table I dissolved in water, JP-1, and JP-2 (JP-1 and JP-2 are acidic, pH 1.2, and neutral, pH 6.8, solutions described in The Pharmacopoeia Japan XII, respectively). All renin inhibitors listed in Table II dissolved slightly in JP-1 but much less in water and JP-2. Although renin inhibitors dissolve relatively well in acidic solution, their oral absorption is expected to be poor because of precipitation in the small intestine at neutral pH.

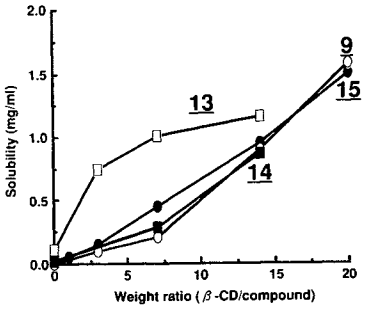


Figure 1 The effect of β-CD on the solubility of renin inhibitors at 25°C.

Solubilizers were examined to improve the low solubility of renin inhibitors in the neutral pH region, and CDs were good solubilizers for renin inhibitors. All CDs in Table III increased the solubility of renin inhibitors in water, while β-CD was most effective. The solubility of renin inhibitors increased with increasing β-CD concentration, but individual profiles were different. Although the complex formation between renin inhibitor and β-CD is not clear at present, this variety of pattern may result from differences in complex solubility and ratio between renin inhibitor and β-CD.

Intestinal Absorption–*in Situ* Loop Method

The absorption of renin inhibitors was evaluated by an *in situ* loop method using the rat small intestine. The cumulative amount (% of dose) of renin inhibitor transported from the loop into the mesenteric vein was used as an index of the absorption. By this index, the absorption from the small intestine could be directly evaluated without hepatic extraction or distribution to tissues.

The metabolic stability of renin inhibitors (compounds *9* and *15*) was checked in rat plasma and 10% intestinal homogenate. Both compounds were stable and degraded by only 10% in 1 hour at 37°C. Renin inhibitors incorporating the statine moiety were reported to be stable toward cleavage *in vivo* (5).

Figure 2 shows the relationship between the solubility of renin inhibitor with β-CD and the cumulative amount (%) transported by 60 min. For all compounds, cumulative transported amounts tended to increase with increasing solubility by addition of β-CD. Transport of compound, *9*, saturated at

Table III. Effect of CDs on Solubility of Renin Inhibitors

Compound	Solubility (mg/ml, 25°C)				
	None[a]	α-CD[b]	β-CD[b]	γ-CD[b]	Hydroxypropyl-β-CD[b]
7		0.04	0.68	0.01	0.29
13	0.108	0.38	1.16	0.47	0.84
14	0.006	0.05	0.77	0.07	0.28
15	0.002	0.03	0.96	0.06	0.24
18	<0.01	<0.01	0.38	0.01	0.02
CH_3-18	0.06	0.21	1.47	0.32	0.84

[a] This column shows the solubility in water without CD.
[b] CD was added at the weight ratio of CD/compound = 14.

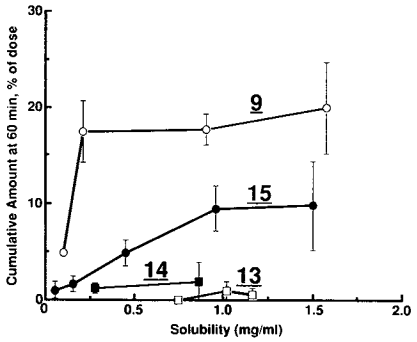


Figure 2 The relationship between the cumulative amount (% of dose) transported within 60 min and the solubility of renin inhibitors. The cumulative amount was measured by the rat *in situ* loop method. The suspension of renin inhibitor (1.5 mg/ml) containing β-CD at an appropriate weight ratio to renin inhibitor, 0.8 ml, was injected directly into the lumen of the ligated intestinal loop. All mesenteric venous blood from the loop was collected continuously at 1-min intervals for 60 min from mesenteric venous cannula. Each value is the mean ± S.D. (n = 3).

a relatively low solubility range. On the other hand, the less-transported compounds, *13* and *14*, showed little increase in cumulative transported amount in spite of their high solubility. The behavior of compound *15* was intermediate. Transport of compounds, *9* and *15*, saturated at different concentrations. The well-transported compound, *9*, saturated at a lower concentration than compound *15*. While the stability of complex between β-CD and renin inhibitor may influence the transport of renin inhibitor from the small intestine, *9* and *15* have similar solubility profiles (Fig. 1) and their stability constants are presumed to be similar. Further, the renin inhibitor itself is thought to be transported from the small intestine but not the complex.

Figure 3 shows the relationship between the absorbability and the molecular structure of renin inhibitors on the basis of the cumulative amount (%) transported within 60 min. Active inhibitors of human plasma renin (their inhibitory concentrations (IC_{50}) were lower than 50nM) were screened. Most were not transported into mesenteric venous blood, with cumulative transported amounts less than 7%. However, the compounds with both a pyridyl group at the C-terminal position and a morpholino group at the N-terminal position, shown by (C + N), were well transported from the intestinal loop i.e., compounds, *9*, *17*, and *18*. However, compounds which had either group at the C-terminal, (C), or at the N-terminal, (N), were poorly transported. In addition, the cumulative transported amount increased by changing the phenyl group to a naphthyl group on the N-terminal side as seen for compounds, *14* to *15* and *17* to *18*.

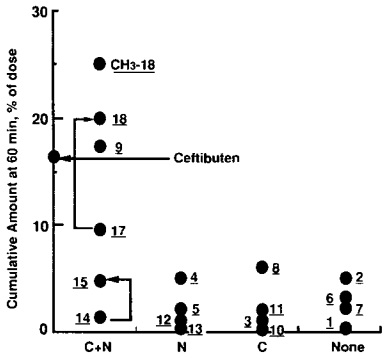


Figure 3 The relationship between the cumulative amount (% of dose) at 60 min and the molecular structure of renin inhibitor. C shows pyridyl group at the C-terminal position and N shows morpholino group at the N-terminal position of renin inhibitor. The cumulative amount of *16* was not measured by its low activity to human plasma renin. The arrow means the change from the phenyl group to the naphthyl group. The mean values (n = 3) were used as data. The suspension (0.8 ml) of renin inhibitor with β-CD (renin inhibitor 1.5 mg/ml, 7 fold β-CD) was injected into the lumen of the ligated intestinal loop.

The substitution effects suggest that structural characteristics affect the transport from the small intestine in addition to solubility. Further, carrier systems in the small intestine may be involved in the absorption of some renin inhibitors. The possibility of carrier system is also suggested by the saturation in the cumulative transported amount of the compounds, *9* and *15*. However, the effect of the naphthyl group on the N-terminal side on the transport is not clear at present.

Compounds *9* and *18* were expected to be fairly well absorbed orally when compared with ceftibuten (orally well-absorbed antibiotic with a bioavailability of 74% (urinary recovery) in healthy human and 57% in rat (21)) for which the cumulative transported amount was 16%.

Intestinal Absorption–Oral Administration

Oral absorption was studied for *9* and *18*, which were well transported from the small intestinal loop (Fig. 3). Both compounds showed low AUCs in spite of the high cumulative amounts (Fig. 4). No trace was detected in plasma in the case of *18* (B.A. c.a. 0%). Although *9* showed high absolute bioavailability (B.A. 42 ± 15% (mean ± S.D., n = 3)), its plasma concentration was low because of rapid blood clearance. However, the higher absolute bioavailability of *9* than *18* might result from differences of *in vivo* stability since *18* had one more amide bond susceptible to peptidases than *9*.

To clarify the discrepancy between the high cumulative amounts and the low AUCs of both compounds, the hepatic first pass effects were compared between two compounds,

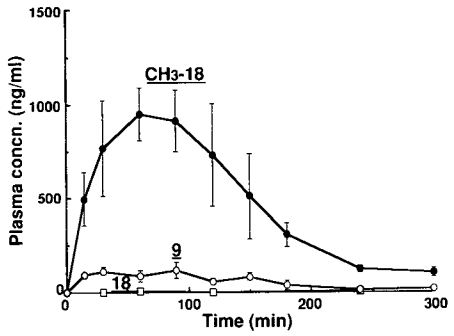


Figure 4 Plasma concentrations of *9*, *18*, and *CH_3-18* after oral administration of their suspension with β-CD (5 mg/ml/rat, 7 fold β-CD). Each value shows the mean ± S.D. (n = 3 – 5). B.A.s were estimated using $AUC_{(0-5\ hr)}$s calculated according to the trapezoidal rule.

Table IV. Hepatic First Pass Effect on Compounds *18* and *CH_3-18*

Compound[a]	Dose (mg/rat)	AUC_{0-2hr} (ng · hr/ml) Portal	Caudal	HER (%)[b]
I8	0.5	103 ± 29	314 ± 65	67.3
CH_3-18	0.5	347 ± 38	358 ± 59	3.1

[a] Each compound dissolved in 5% Tween 80 saline (500 μl) was infused for 15 min.

[b] HER; Hepatic Extraction Ratio, HER = (1-AUCportal/AUCcaudal) × 100. The data were expressed as mean ± SD (n = 3).

18 and *CH_3-18*. *CH_3-18* which was intended to evade the hepatic first pass effect, was N-methylated at thiazolyl alanine of *18*, and its cumulative amount was 25 ± 4% (mean ± S.D., n = 3)) by 60 min. As Table IV shows, *18* had higher hepatic first pass extraction (67%) than *CH_3-18* (3%). The plasma concentration of *CH_3-18* after oral administration was the highest of the three compounds, and its AUC increased dramatically by N-methylation (Fig. 4). The absolute bioavailability of *CH_3-18* was 22 ± 2% (mean ± S.D., n = 5). These findings show that the low AUC of *18* was caused by a strong hepatic first pass effect.

We conclude that the use of β-CD and the proper terminal substituents could increase the intestinal absorption of renin inhibitors, and N-methylation at the amide bond could suppress the hepatic first pass effect. Therefore, it may be possible to synthesize a new renin inhibitor which is better absorbed than presently available compounds and is resistant to the hepatic clearance. Since some renin inhibitors are suggested to be absorbed from the small intestine by a carrier system (22), the utilization of this system may be possible.

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