ES-8891, an Orally Active Inhibitor of Human Renin

Tatsuo Kokubu, Kunio Hiwada, Eiki Murakami, Shinjiro Muneta, Yutaka Kitami, and Patrick F. Salmon

A newly synthesized orally active renin inhibitor, N-morpholinoacetyl-(1-naphthyl)-L-alanyl-(4-thiazolyl)-L-alanyl (3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoyl-n-hexylamide (ES-8891), was found to be a highly potent competitive inhibitor of human renin with an inhibition constant of 1.1 nM. This inhibitor was also active against monkey renin, although there was less inhibition of renin in pig, rabbit, and rat. ES-8891 did not inhibit cathepsin D, pepsin, trypsin, chymotrypsin, angiotensin converting enzyme, and urinary kallikrein at a concentration of 10^{-5} M. A single oral administration of ES-8891 (10 or 30 mg/kg) to conscious, sodium-depleted marmosets caused a dose-related decrease in plasma renin activity and blood pressure. ES-8891 (30 mg/kg) produced an 80% inhibition of plasma renin activity, which lasted for more than 6 hours. Kidney renin messenger RNA was not significantly changed 6 hours after oral administration of ES-8891 (30 mg/kg). A single oral administration of 240 mg ES-8891 to healthy human volunteers (n=6) produced a significant inhibition of plasma renin activity (75% inhibition at 0.5 and 1 hour, 50% inhibition at 2 hours) with a good correlation of plasma levels of ES-8891. There were no significant changes in blood pressure or heart rate, and no adverse effects were observed. These results suggest that ES-8891 is an orally active human renin inhibitor that may be clinically useful. (Hypertension 1990;15:909-913)

The renin-angiotensin system plays an important role in the regulation of blood pressure and in body fluid homeostasis. Inhibitors of angiotensin converting enzyme (ACE) are now widely used in patients with hypertension and congestive heart failure. Since ACE converts angiotensin I to angiotensin II and also hydrolyzes a variety of biologically active peptides, ACE inhibitors are not a specific blocker of the renin-angiotensin system. However, renin acts on angiotensinogen as its only known substrate. Renin inhibitors may block the renin-angiotensin system in a selective manner and would be expected to produce changes of physiological significance in the renin-angiotensin system. Use of renin inhibitors may also reduce the incidence of ACE inhibitor-induced adverse effects, such as dry cough or angioedema, which may be independent of the renin-angiotensin system. Recently, highly potent competitive inhibitors of human renin have been synthesized and have been demonstrated to be effective after oral administration in animals. However, because of their low bioavailability, doses of more than 10 mg/kg were required to cause a significant decrease in plasma renin activity (PRA). At present, no effective compound based on renin inhibition is available for use as an antihypertensive drug. The present study describes an orally active renin inhibitor-containing statine analogue, N-morpholinoacetyl-(1-naphthyl)-L-alanyl-(4-thiazolyl)-L-alanyl (3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoyl-n-hexylamide (ES-8891), and documents its effect on PRA after oral administration to normotensive volunteers.

Methods

Human Renin Inhibitor ES-8891

ES-8891 was synthesized by Sankyo Company (Tokyo, Japan). The structure is shown in Figure 1 and is compared with ES-6864 (reported in our previous paper). The molecular weight of ES-8891 is 795, and the partition coefficient (log P) between 1-octanol and simulated gastric juice (distilled water with pH 1.2) is 2.6.

In Vitro Study

Renin activity in the presence and absence of the inhibitor was measured by a previously reported method. The inhibition constant (K) for ES-8891 was determined by using the plot of Dixon and
The hybridization study was carried out as described by the method of slot-blot hybridization. This synthetic renin mRNA fragment was used for the synthesis of renin mRNA fragment by in vitro transcription from the T7 RNA polymerase promoter. This synthetic renin mRNA fragment was used as a standard for the native renin mRNA. The quantitative analysis of the kidney renin mRNA was carried out by the method of slot-blot hybridization assay. Serial dilutions of the kidney total RNA were used for analysis of renin mRNA and $\beta$-actin mRNA. The hybridization study was carried out as described previously.

Plasma levels of ES-8891 were measured after an oral administration of ES-8891 to the marmosets (3 or 10 mg/kg). Plasma concentration of the compound was determined by high-performance liquid chromatography with a fully automated column-switching technique.

**Human Study**

Eight healthy male volunteers aged 22–29 years, without metabolic, endocrine, hepatic, renal, or cardiovascular abnormalities, received single ascending oral doses of 60, 120, and 240 mg ES-8891 or placebo, with a washout period of at least 7 days between successive doses. There was no extreme variation in height or weight among the subjects. The study was performed in accordance with the declaration of Helsinki and was approved by a properly constituted ethics committee. Informed consent was obtained from each subject before entry into the study. At each dose level, the same six subjects received the active drug, and the same two received the placebo. Progression to each higher dose depended on the assessment of tolerance of the previous dose. Subjects were admitted approximately 24 hours before drug administration and remained under observation for at least 24 hours after each dose, when routine laboratory assessments were repeated. During the study, vital functions and electrocardiograms were recorded in the resting state before dosing and at 0.5, 1, 2, 3, 5, 8, and 24 hours after dosing. After an overnight fast of at least 8 hours, a single oral dose of ES-8891 was administered, and the subjects continued to fast for a further 5 hours. All subjects remained in bed with minimal activity during this period and were supine for at least 20 minutes before the collection of blood samples. Salt intake was not restricted, although daily salt intake was recorded. Blood samples were obtained before and at 0.5, 1, 2, 3, 5, 8, and 24 hours after each dose for measurement of PRA, plasma angiotensin I, and ES-8891 levels. Fractionated urine collection for determination of urinary sodium, potassium, and chloride was also made.

**Statistical Analysis**

Results are expressed as mean±SEM. The Mann-Whitney U test was used to test for differences among groups of means. Wilcoxon's $t$ test was used to test for differences between any two means within a group. Differences were considered significant at values of $p<0.05$.

**Results**

**In Vitro Study**

ES-8891 inhibited human renin with an IC$_{50}$ of $1.1 \times 10^{-9}$ M. The $K_I$ value of ES-8891 for human renin and human angiotensinogen was $1.1 \times 10^{-9}$ M. The mode of inhibition proved to be competitive. Table 1 shows the inhibitory effect of ES-8891 on renin from humans and animals. The compound was similarly potent against monkey renin but was considerably less active against renin from rabbit and pig and was very weak against rat renin. The inhibitory effect of ES-8891 on aspartyl proteases, such as

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**Figure 1. Schematic representation of chemical structures of renin inhibitors ES-8891 and ES-6864. For discussion, see Reference 4.**
cathepsin D and pepsin, as well as other proteases was also studied. The compound did not inhibit human cathepsin D, human pepsin, bovine trypsin, bovine chymotrypsin, human ACE, or human urinary kallikrein at a concentration of $10^{-5}$ M.

**TABLE 1. Inhibitory Effect of ES-8891 on Renin From Humans and Five Different Species of Animal**

<table>
<thead>
<tr>
<th>Renin</th>
<th>Angiotensinogen</th>
<th>IC$_{50}$ ($\times 10^{-4}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (kidney)</td>
<td>Human</td>
<td>1.1</td>
</tr>
<tr>
<td>Human (plasma)</td>
<td>Endogenous</td>
<td>2.6</td>
</tr>
<tr>
<td>Monkey</td>
<td>Sheep</td>
<td>2.6</td>
</tr>
<tr>
<td>Dog</td>
<td>Pig</td>
<td>4.1</td>
</tr>
<tr>
<td>Pig</td>
<td>Pig</td>
<td>25</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Rabbit</td>
<td>31</td>
</tr>
<tr>
<td>Rat</td>
<td>Rat</td>
<td>140</td>
</tr>
</tbody>
</table>

Each value is the mean of three measurements. IC$_{50}$, 50% inhibitory concentration.

Study in Marmosets

Figure 2 shows a dose-dependent decrease in PRA and blood pressure by a single oral administration of ES-8891 to marmosets. One and 6 hours after oral administration of ES-8891 (30 mg/kg), PRA was inhibited by 90% and 80%, respectively. There was a slight dissociation between the level of PRA and blood pressure response to renin inhibition. Mean blood pressure decreased significantly at doses of 10 and 30 mg/kg. At a dose of 30 mg/kg, lowest mean blood pressure was 89±8 mm Hg compared with a mean blood pressure of 108±8 mm Hg before administration. The blood pressure reduction persisted for 6 hours. There was no significant change in heart rate. The vehicle (sterile water) did not affect either blood pressure or heart rate. There was no significant difference in the relative amounts of $\beta$-actin mRNA between the control marmosets and marmosets treated with ES-8891 (30 mg/kg) at 6 hours after oral administration. The kidney renin mRNA contents of the control and ES-8891–treated marmosets were 10.7±2.4 and 10.3±1.0 pg/fig total RNA, respectively. There was no significant difference in the amounts of kidney renin mRNA between the two groups.

After an oral administration of ES-8891, the absorption was rapid with a peak time of 0.5 hour. The peak concentrations for 3 mg/kg and 10 mg/kg doses were 26.0±13.8 (n=3) and 57.2±18.3 (n=5) ng/ml, respectively, and the absorption indexes (areas under the curve) were 49.5±24.5 and 122.7±74.5 ng/hr/ml, respectively.

**Human Study**

ES-8891 was well tolerated in healthy volunteers at the administered doses. No clinically significant changes in vital functions or in electrocardiographic records were noted during the study. No adverse effects attributable to ES-8891 were observed in hematologic or biochemical parameters. There was no significant change in urinary sodium, potassium, or chloride in the 24 hours after each dose. Figure 3 shows plasma concentrations of ES-8891 and PRA after oral administration of 240 mg ES-8891 to six human volunteers. PRA was decreased rapidly with
an associated increase in plasma ES-8891 levels and returned gradually to predose levels; this return correlated with the disappearance of the compound from plasma over a period of 5 hours. Twenty-four hours after the 240 mg dose, PRA was also noted to be significantly elevated compared with predose levels. A dose-relation for PRA suppression was not observed in the doses of ES-8891 (60, 120, and 240 mg). Plasma angiotensin I at 0.5 hour after ES-8891 administration (240 mg) tended to decrease compared with the predose value (0.26±0.05 vs. 0.38±0.10 ng/ml, respectively), but this difference did not achieve statistical significance. There was no clinically significant or consistent change in blood pressure or heart rate at any time during the study. After each dose, the absorption speed was rapid with a peak time of 0.5 hour; peak concentrations at that time were 8.5±3.1, 15.1±2.4, and 42.4±7.6 ng/ml for the 60-, 120-, and 240-mg doses, respectively; these values indicated an almost linear relation between dose and peak concentration. A linear relation was also noted between absorption index and the dose. The absorption index after 240 mg was 98.5±33.5 ng/hr/ml (n=6). An elimination rate, calculated as half-life, was 0.95±0.31 hours (n=6) for the 240-mg dose.

Discussion

The success of ACE inhibitors in the treatment of hypertension has stimulated the development of specific inhibitors of human renin. Hemodynamic and biochemical consequences of renin inhibition have been studied after parenteral administration in normal humans,19-22 but no orally active renin inhibitor is yet available. The major problems encountered have been low oral bioavailability and an insufficient duration of action. This study demonstrates that ES-8891 is an orally active renin inhibitor that is potent and highly species- and enzyme-specific for human renin. The in vitro results demonstrated that ES-8891 is a more potent inhibitor of human renin than ES-6864, as reported previously.4 Replacement of morpholine residue by hexylamide at the C-terminus of ES-6864 renders the resulting compound more potent and less toxic.

The extensive clinical and laboratory assessments evaluated in the present study demonstrated that doses of 60, 120, and 240 mg ES-8891 were very well tolerated in humans and that ES-8891 proved to be a potent inhibitor of PRA at these dose levels. However, plasma angiotensin I concentration was not significantly suppressed even at the 240 mg dose. The in vitro measurement of PRA may overestimate the in vivo blockade of renin. PRA was high 24 hours after administration of ES-8891; thus, renin release is still stimulated after the renin inhibitor has been metabolized.

The absorption of ES-8891 was similar in marmosets and in humans. The bioavailability of ES-8891 could not be estimated because the compound could not be dissolved in nontoxic solvents for intravenous administration. Comparison of the absorption indexes of the two compounds indicates that the bioavailability of ES-8891 is 10 times greater than that of renin inhibitor CGP 38560A.22

In this study, blood pressure in the volunteers did not fall despite the decrease in PRA; this was in accordance with previous results obtained after intravenous infusion of renin inhibitors (enalkiren and CGP 38560A) in normal subjects.20-22 This suggests that maintenance of blood pressure in normal sodium-unrestricted subjects is less dependent on the renin-angiotensin system.23

Nakamura et al24 have reported that renin mRNA is increased significantly with an elevation of PRA after treatment with ACE inhibitors. We recently reported that continuous intravenous infusion of the renin inhibitor ES-1005 suppressed the expression of kidney renin mRNA in marmosets.31 In the present study, we did not observe a significant change in kidney renin mRNA after oral administration of ES-8891 in that species. The detailed mechanism of action of ES-1005 or ES-8891 in kidney renin gene expression is unknown.

In conclusion, ES-8891 is an orally active inhibitor of human renin with high potency and specificity and with no adverse effects in humans. The results suggest that ES-8891 may be useful in clinical study.

References


**KEY WORDS** • renin inhibitor • blood pressure • plasma renin activity • monkey studies • human studies • renin
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