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.

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 ($K_i$) for ES-8891 was determined by using the plot of Dixon and
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.17

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 Helsinki18 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 IC50 of 1.1×10−9 M. The K value of ES-8891 for human renin and human angiotensinogen was 1.1×10−9 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

Study in Marmosets
Marmosets of either sex weighing 250–300 g were fed a low sodium diet for 1 week. The methods for recording blood pressure and heart rate have been described previously.12 Cathepsin D, pepsin, trypsin, chymotrypsin, ACE, and urinary kalikrein were measured in the presence and absence of ES-8891 with the corresponding substrates as described previously.12 Protein was measured by the method of Lowry et al,13 with bovine serum albumin used as a standard.

Webb.11 Human PRA and marmoset PRA were measured as described previously.12 Cathepsin D, pepsin, trypsin, chymotrypsin, ACE, and urinary kalikrein were measured in the presence and absence of ES-8891 with the corresponding substrates as described previously.12 Protein was measured by the method of Lowry et al,13 with bovine serum albumin used as a standard.

The hybridization study was carried out as described previously.15 A 420-base pair fragment of human renin cDNA was cloned into the multiple cloning site of vector pT3T7 lac (Boehringer Mannheim, Mannheim, FRG). The resulting plasmid 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 β-actin mRNA. The hybridization study was carried out as described previously.15

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.17

FIGURE 1. Schematic representation of chemical structures of renin inhibitors ES-8891 and ES-6864. For discussion, see Reference 4.
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>IC₅₀ (×10⁻⁵ 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. IC₅₀, 50% inhibitory concentration.

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.

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 β-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/mg 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

![Graph showing plasma renin activity (PRA) and mean arterial blood pressure (BP) as a function of time and of oral dose of renin inhibitor ES-8891 in sodium-depleted conscious marmosets. Ang I, angiotensin I; , 10 mg/kg ES-8891 (n=5); , 30 mg/kg ES-8891 (n=3); , vehicle (n=5). Values are mean±SEM (bars). *Significant reduction of PRA compared with pretreatment value (p<0.05).](http://hyper.ahajournals.org/lookup/doi/10.1161/01.HYP.911.4.911)
an associated increase in plasma ES-8891 levels and
returned gradually to predose levels; this return
related 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 lev-
ses. 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 com-
pared 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 spe-
cific inhibitors of human renin. Hemodynamic and
biochemical consequences of renin inhibition have
been studied after parenteral administration in nor-
mal 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. How-
ever, 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 marmo-
sets 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 intra-
venous 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.14 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 sug-
gest that ES-8891 may be useful in clinical study.

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Kokubu et al  Orally Active Renin Inhibitor  913


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