Fate of Recombinant Human Renin Administered Exogenously to Anesthetized Monkeys

MASAHITO HIRUMA, SHOKEI KIM, FUMIHIKO IKEMOTO, KAZUO MURAKAMI, AND KENJIRO YAMAMOTO

SUMMARY Highly purified recombinant human renin (rh-renin), synthesized by Chinese hamster ovary cells, was labeled with iodine-125 and was given intravenously to pentobarbital-anesthetized common marmosets (Callithrix jacchus) to study the fate of the circulating renin. Specific anti-rh-renin antiserum was used to identify the 125I-rh-renin. Plasma disappearance of the exogenously administered 125I-rh-renin in marmosets (n = 6) showed two exponential components, with a half-life of 12.1 ± 1.9 minutes for the rapid component and 120.3 ± 16.4 minutes for the slow component. The metabolic clearance rate was 1.17 ± 0.26 ml/min/kg. Thirty minutes after the injection of 125I-rh-renin, 43.1 ± 0.9 and 3.5 ± 0.5% of the injected dose had distributed to the liver and the kidneys, respectively. With time, the accumulated 125I-rh-renin in the liver and kidneys decreased. The accumulation of 125I-rh-renin was less than 1% of the dose injected in other organs such as lungs, heart, spleen, adrenal glands, testes, and ovaries. Analysis of liver and kidney extracts by high performance liquid chromatography at 30 and 120 minutes indicated that immunoreactive 125I-rh-renin decreased with time and was accompanied by an increase in nonimmunoreactive degradation products of a low molecular weight. The incubation of 125I-rh-renin with monkey or human plasma at 37 °C did not degrade the labeled renin. Therefore, rh-renin was rapidly cleared from the circulation by the liver and the kidney.

KEY WORDS • recombinant human renin • renin clearance • monkey studies • liver • kidney

THE plasma level of renin, a key enzyme of the renin-angiotensin system,1 is regulated by the rate of release from the kidney and by plasma clearance. We studied the plasma clearance of exogenously administered mouse submaxillary renin in mice and rat renal renin in rats, both labeled with iodine-125 and found that mouse submaxillary renin is cleared mainly by the kidney,2-5 while rat renal renin is cleared by the liver as well as by the kidney.6-9 We suggested the possible contribution of glycosylation of renal renin to the hepatic distribution.6-9 However, there is a significant difference in the characteristics of the renin-angiotensin system between human renin and laboratory animal renins, including dog, hog, rat, and mouse renin.10-13 Specific antibodies against human renin only weakly cross-react with renin from these animal species.10 Human renin can catabolize angiotensinogen from other species, while laboratory animal renin cannot catabolize human angiotensinogen.11 Renin inhibitors specific for human renin are not potent against renin from various other species.12, 13 Thus, the clearance of human renin should be studied using primates.

In the present study, highly purified active recombinant human renin (rh-renin), synthesized by Chinese hamster ovary cells transfected with a plasmid containing human renin complementary DNA (cDNA),14, 15 was labeled with Iodine-125 and given intravenously to monkeys to study the fate of this renin.

Materials and Methods

Animals

Female cynomolgus monkeys (Macaca fascicularis; CLEA Japan, Tokyo, Japan), weighing 2.5 to 3.0 kg, were used to study the pressor activity of rh-renin. Common marmosets (Callithrix jacchus; CLEA Japan) of both sexes, weighing 250 to 350 g, were used to study the clearance of rh-renin given exogenously. The animals were kept in separate cages and at a constant temperature (23 °C). All...
were fed a standard monkey diet (CMK-1; CLEA Japan) fruit, and tap water, ad libitum.

Preparation of Recombinant Human Renin

rh-Renin, expressed in Chinese hamster ovary cells transfected with a plasmid containing the human renin cDNA, and activated with trypsin for 2 hours at 24 °C, was purified to apparent homogeneity, as described by Poorman et al. and Murakami et al. rh-Renin is similar to native human renal renin with regard to physiochemical characteristics.

Pressor Activity of Recombinant Human Renin

Experiments were performed on pentobarbital-anesthetized cynomolgus monkeys (25 mg/kg i.v.). A femoral artery was catheterized with a polyvinyl tube for the measurement of blood pressure and heart rate and for the collection of blood samples. A femoral vein was cannulated for the injection of substances. At specified times after the injection of rh-renin, arterial blood (3–5 ml) was collected for the measurement of angiotensins I and II.

Measurement of Plasma Angiotensins I and II

The levels of plasma angiotensins were routinely determined by the method of Kawamura et al. Arterial blood collected from cynomolgus monkeys into a chilled tube containing Na2H-EDTA powder was immediately centrifuged at 3000 rpm for 15 minutes at 4 °C. Then, 2 ml of plasma was applied to Sep-Pak cartridges (Waters Associates, Milford, MA, USA), and the bound angiotensins were eluted with 3 ml of methanol/water/trifluoroacetic acid (80:19.9:0.1, vol/vol/vol). The eluates were then applied to a reverse-phase column (TSK gel ODS-80TM, 4.6 × 150 mm; Toyo Soda, Yamaguchi, Japan) and eluted with a 15-minute linear gradient of 10 to 50% methanol at a flow rate of 0.8 ml/min to separate angiotensins I, II, and III. The retention time of angiotensin I, II, and III standards was 27.3, 24.4, and 23.0 minutes, respectively. Radioimmunoassay (RIA) for angiotensin II was performed using specific antiserum against angiotensin II, a gift from Dr. K. Shimamoto. Sensitivity of the assay was 0.2 pg of angiotensin II. RIA for angiotensin I was performed using specific antiserum against angiotensin I, as reported.

Iodination of Recombinant Human Renin

rh-Renin was labeled with Iodine-125 by the chloramine-T method, as reported. First, 1 mcCi of Na125I and 15 μg of rh-renin were allowed to react with 100 μg of chloramine-T in 140 μl of 0.1 M sodium phosphate buffer (pH 7.4) for 15 seconds at room temperature (20–25 °C). Then, 200 μg of sodium metabisulfite in 50 μl of 0.1 M sodium phosphate buffer (pH 7.4) was added to halt the reaction. The reaction mixture was then applied to a Sephadex G-25 column equilibrated with 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl and 0.3% bovine serum albumin (BSA) at a flow rate of 0.5 ml/hr, with fractions of 0.8 ml being collected. 125I-labeled rh-renin, eluted in the void volume from this column, was then applied to a concanavalin A-Sepharose column (7 × 20 mm) equilibrated with 20 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl at room temperature. The column was exhaustively washed with the equilibration buffer and then eluted with 50 mM a-methyl-d-mannoside in the equilibration buffer, with 0.5-ml fractions being collected. This 125I-rh-renin was further purified by gel permeation high performance liquid chromatography (HPLC) on a G 3000 SW column (Toyosoda) equilibrated with 20 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl.

Determination of Immunoreactive 125I-rh-Renin

Rabbit-specific anti-rh-renin antiserum was used to detect 125I-rh-renin in samples of plasma, urine, and liver and kidney extracts. This antiserum completely cross-reacted with native human kidney and plasma renin. The 50% inhibitory concentrations of this antiserum for pure native human renal renin and plasma renin were 7.5 × 10⁻⁴ and 2 × 10⁻⁴, respectively. Nonimmunized rabbit serum was used as the control. For plasma samples, 25 μl of plasma was added to 475 μl of the antiserum, diluted with 50 mM phosphate buffer (pH 7.4) containing 0.1 M NaCl and 0.3% BSA (1:2500) and was incubated for 48 hours at 4 °C. Then, 100 μl of goat antirabbit Immunoglobulin G antiserum diluted with the same buffer (1:200) was added and the preparation further incubated for 24 hours at 4 °C, followed by centrifugation at 3000 rpm for 20 minutes at 4 °C, for the separation of antibody-bound and free radioactivities. Both precipitate and supernatant were counted for 125I and 131I radioactivities.

For liver extract and urine and for kidney extract, 25- and 15-μl aliquots, respectively, were incubated with specific antiserum or nonimmunized serum, as described. After incubation for 24 hours at 4 °C, 100 μl of 1.6% rabbit γ-globulin (final concentration, 0.1%) and 1 ml of 20% polyethylene glycol 6000 (final concentration, 12.5%) were added to the incubation mixture, followed by centrifugation at 3000 rpm for 15 minutes at 4 °C, and the radioactivity in both supernatant and precipitate was measured. In all assays, the 125I radioactivity precipitable with specific antiserum was corrected for that seen with nonimmunized rabbit serum. Nonspecific binding with nonimmunized rabbit serum was only 2 to 5% throughout the assays. Thus, 125I-rh-renin in each sample could be specifically measured using this immunoprecipitation method.

Plasma Clearance of 125I-rh-Renin in Marmosets

Marmosets were anesthetized with ketamine (10 mg/kg i.m.), and polyethylene tubes were inserted into the left femoral artery and vein for blood sampling and for the injection of 125I-rh-renin, respectively. The pentobarbital-anesthetized animals (20 mg/kg i.v.) were given a bolus injection of 7.7 to 9.0
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Organ Distribution of 125I-rh-Renin in Marmosets

Organ distribution of 125I-rh-renin was studied 30 and 120 minutes after the intravenous injection of 125I-rh-renin (7.7–9.0 × 10^6 cpm) and 131I-labeled human serum albumin (2.0 × 10^6 cpm). Immediately after the blood sampling at 30 or 120 minutes, the animals were killed by a bolus injection of pentobarbital and organ distributions of 125I-rh-renin at 120 minutes were studied.

The plasma was separated from the whole blood by centrifugation at 3000 rpm for 20 minutes at 4 °C. An aliquot of 25 μl of each plasma sample was used for the determination of immunoreactive 125I-rh-renin, as described. For each animal, the radioactivity of immunoreactive 125I-rh-renin in the plasma was plotted semilogarithmically against time. Plasma disappearance curves of immunoreactive 125I-rh-renin were analyzed according to a two-compartment exponential model, as reported. Lines of best fit were determined by the nonlinear least-squares method.

Organ Distribution of 125I-rh-Renin in Marmosets

Organ distribution of 125I-rh-renin was studied 30 and 120 minutes after the intravenous injection of 125I-rh-renin (7.7–9.0 × 10^6 cpm) and 131I-labeled human serum albumin (2.0 × 10^6 cpm). Immediately after the blood sampling at 30 or 120 minutes, the animals were killed by a bolus injection of pentobarbital. The urine in the bladder was collected with a syringe, and heart, lung, liver, kidneys, adrenal glands, testes or ovaries, and spleen were removed. Each organ was rinsed with cold saline, weighed, and counted for 125I and 131I radioactivities.

Liver and kidneys were homogenized with cold phosphate buffer (pH 7.4) using a glass homogenizer (1:10, wt/vol). 125I-rh-renin was not degraded by the homogenization procedure, as demonstrated in preliminary experiments in which the 125I-rh-renin was not catabolized when incubated with liver or kidney homogenates at pH 7.4 for 3 hours at 37 °C or for 72 hours at 4 °C. The supernatant was separated from the whole homogenate by centrifugation at 105,000 g for 60 minutes at 4 °C.

The level of 131I radioactivity in an organ was assumed to reflect contamination with blood of the injected albumin. With 131I-labeled albumin as a plasma marker, the amount of 125I radioactivity in the plasma in each organ was calculated, and this value was subtracted from the total amount of 125I radioactivity in each organ to give the distribution of 125I radioactivity.

Incubation of 125I-rh-Renin with Plasma

The labeled renin (2 × 10^6 cpm) was incubated with 1 ml of monkey or human plasma for 1, 3, or 7 hours at 37 °C. The incubated sample (500 μl) was applied to a G 3000 SW column at a flow rate of 0.5 ml/min at room temperature, with fractions of 0.5 ml being collected. Total radioactivity and radioactivity precipitable with specific antirenin antibody in each fraction were counted.

Molecular Weight Determination

The molecular weight of radioactive substances in plasma, liver and kidney extracts, and urine was measured by HPLC on a G 3000 SW column equilibrated with PBS at a flow rate of 0.5 ml/min at room temperature. Fractions of 0.5 ml were collected. The 125I-rh-renin in each fraction was measured using the immunoprecipitation technique described.

Measurement of Radioactivity

125I and 131I radioactivities were assayed with a two-channel γ-counter (Packard Model Auto Gamma 500 C Counting System, Rockville, MD, USA). Corrections were made for isotope spillover, decay, and counting efficiency. Spillover of 131I into the 125I channel was 15%. There was no spillover of 125I into the 131I channel. 125I and 131I radioactivities were counted within a 1% error.

Results

Pressor Activity of Recombinant Human Renin

As shown in Figure 1, the intravenous administration of rh-renin (1 μg/kg) to an anesthetized cynomolgus monkey led to a 31 ± 5 mm Hg increase in mean blood pressure associated with an increase in plasma angiotensin I (Ang I) and II (Ang II) concentrations. Pretreatment with captopril (1 mg/kg i.v.) completely blocked the pressor activity. Values are means ± SE.

![FIGURE 1. Pressor activity of recombinant human renin (rh-renin). The intravenous administration of rh-renin (1 μg/kg) to an anesthetized cynomolgus monkey led to a 31 ± 5 mm Hg increase in mean blood pressure associated with an increase in plasma angiotensin I (Ang I) and II (Ang II) concentrations. Pretreatment with captopril (1 mg/kg i.v.) completely blocked the pressor activity. Values are means ± SE.](http://hyper.ahajournals.org/)
Iodination of Recombinant Human Renin

As shown in Figure 2A, most of the $^{125}$I-rh-renin applied was adsorbed to a concanavalin A column and eluted with 50 mM $\alpha$-methyl-$D$-mannoside, thereby indicating the glycosylation of this rh-renin. Rechromatography of this purified $^{125}$I-rh-renin on a G 3000 SW column showed a single symmetrical peak with a retention time of 39.0 minutes, corresponding to a molecular weight of 42,000 (Figure 2B). $^{125}$I-rh-renin retained the immunoreactivity and pressor activity, thereby indicating little iodination damage. $^{125}$I-rh-renin was stored in PBS containing 0.5% BSA at 4°C and used within 1 week after iodination, during which time the immunoreactivity and pressor activity remained unchanged. In addition, BSA did not bind to this labeled renin.

Plasma Disappearance of $^{125}$I-rh-Renin

As shown in Figure 3, the disappearance of immunoreactive $^{125}$I-rh-renin from the circulation could be resolved into two exponential components, the half-life for the rapid phase and for the slow phase being 12.1 ± 1.9 and 120.3 ± 16.4 minutes (means ± SE), respectively, and the metabolic clearance rate being 1.17 ± 0.26 ml/min/kg ($n = 6$).

The HPLC profile of the plasma, as shown in Figure 4, represented two major peaks, including the immunoreactive $^{125}$I-rh-renin and nonimmunoreactive degradation products, with a retention time of 39.0 and 47.0 minutes, respectively. With time, immunoreactive $^{125}$I-rh-renin decreased while the degradation products with a low molecular weight increased.

In Vitro Incubation of $^{125}$I-rh-Renin with Plasma

As shown by the HPLC profile in Figure 5, incubation of $^{125}$I-rh-renin with monkey plasma for 3 hours at 37°C resulted in no degradation of the labeled renin and no loss of its immunoreactivity. Similar results were observed during the incubation with human plasma.

Organ Distribution of $^{125}$I-rh-Renin

As shown in Table 1, thirty minutes after the injection, 43.1 ± 0.9 and 3.5 ± 0.5% of the injected dose accumulated in the liver and kidney, respectively, and 52.3 ± 2.6 and 45.4 ± 4.0%, respectively, of the accumulated radioactivities were immunoreactive $^{125}$I-rh-renin ($n = 5$). In the other organs, the HPLC profile of the plasma, as shown in Figure 4, represented two major peaks, including the immunoreactive $^{125}$I-rh-renin and nonimmunoreactive degradation products, with a retention time of 39.0 and 47.0 minutes, respectively.
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TABLE 1. Distribution in Monkeys of Intravenously Administered 125I-Labeled Recombinant Human Renin

<table>
<thead>
<tr>
<th>Organ or fluid</th>
<th>Percentage of dose injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min (n = 5)</td>
</tr>
<tr>
<td>Plasma</td>
<td>29.7 ± 2.5 (80.5 ± 1.2)</td>
</tr>
<tr>
<td>Heart</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Lung</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Liver</td>
<td>43.1 ± 0.9 (52.3 ± 2.6)</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.5 ± 0.5 (45.4 ± 4.0)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Testis or ovary</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Total</td>
<td>78.7 ± 2.6</td>
</tr>
</tbody>
</table>

Values represent means ± SE for each time point. Values in parentheses indicate the percentage of immunoreactive 123I-renin to total 123I.

examined, except for the spleen, less than 1% of the total administered dose was recovered at 30 minutes. At 120 minutes, the accumulated dose in the liver and kidney decreased to 13.1 ± 3.8 and 3.0 ± 0.4%, respectively, and the percentage of immunoreactive 123I-renin in relation to total 123I decreased to 26.1 ± 6.4 and 30.7 ± 3.2%, respectively (n = 6).

The accumulated radioactivities per gram of tissue weight at 30 minutes were 405 ± 79 and 163 ± 29 x 10^3 cpm/g tissue for the liver and kidney and, at 120 minutes, were 101 ± 36 and 137 ± 17 x 10^3 cpm/g tissue for the liver and kidney, respectively. Thus, the amount of accumulated 123I-rh-renin per gram of tissue was similar between the liver and kidney.

Cumulative excretion of 123I-rh-renin into the urine up to 120 minutes was less than 2% of the injected dose (n = 3), thereby indicating the negligible urinary excretion of renin, a finding consistent with the data in the dog24 and rat.6

HPLC profiles of liver and kidney extracts at 30 and 120 minutes showed two major peaks, including immunoreactive 123I-rh-renin and the degradation products with a low molecular weight (Figure 6). At 120 minutes, the 123I-rh-renin peak decreased and was accompanied by an increase in the degradation products.

Discussion

rh-Renin was prepared by trypsin activation of prorenin synthesized by Chinese hamster ovary cells. The rh-renin14, 15 has physicochemical characteristics similar to those of native human renal renin,17 including compositional or N-terminal amino acid sequence, molecular weight, isoelectric point, specific enzymatic activity, and optimum pH against hog angiotensinogen. In addition, as shown in Figure 1, rh-renin had as potent a pressor activity as the native human renin has.23 In the present study, we used as a probe highly purified rh-renin labeled with 123I and specific antiserum. Clearance of exogenously administered rh-renin was studied in the monkey, a species with characteristics of the renin-angiotensin system similar to those in humans. This seems to be a useful model for studying the human renin-angiotensin system.25 In studies of the clearance of circulating renin,26-28 renin was detected in the form of its enzymatic activity. Hence, renin activity is not specific for renin itself; it is affected by various factors in the plasma30.31; and, therefore, is probably an inadequate method for evaluating the clearance of this enzyme.

Plasma disappearance of exogenously administered rh-renin in monkeys showed two exponential components, in agreement with data on the disap-
Fer's cells, and endothelial cells, possess various 
substrates of glycoproteins and participate in the uptake 
receptors that specifically recognize the carbohydrate 
fore confirms the importance of glycosylation of 
distributed in the liver. The present study there-
showed that liver cells, including hepatocytes, Kupf-
tubules, and thereafter is distributed in the lysosomes 
to be degraded. Immunohistochemical findings with specific human renin antiserum showed 
localization of renin in the proximal tubules of the human kidney. Thus, some of the circulating 
human renin may be filtered through glomerular capillaries and reabsorbed by the proximal tubule.

In conclusion, human renin was cleared mainly by the liver, and glycosylation probably contributed to the hepatic clearance.

**Acknowledgments**

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**References**


**Figure 6.** HPLC profile of liver and kidney extracts 30 and 120 minutes after the injection on a G 3000 SW column. Two major peaks were observed, including 125I-rh-renin and the degradation products of low molecular weight. $K = 10^2; V_0 =$ void volume. Open circles indicate total 125I; closed circles indicate 125I-rh-renin.
Fate of recombinant human renin administered exogenously to anesthetized monkeys.
M Hiruma, S Kim, F Ikemoto, K Murakami and K Yamamoto

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