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# HIGHLY POTENT AND SPECIFIC INHIBITORS OF HUMAN RENIN

T. Kokubu, K. Hiwada, Y. Sato, T. Iwata, Y. Imamura, R. Matsueda*, Y. Yabe*, H. Kogen*, M. Yamazaki*, Y. Iijima*, and Y. Baba*

2nd Department of Internal Medicine
Ehime University School of Medicine
Onsen-gun, Ehime 791-02, Japan

*Chemical and Biological Research Laboratories
Sankyo Co. Ltd., Shinagawa-ku, Tokyo 140, Japan

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SUMMARY: We designed aldehyde derivatives of small peptides representing the C-terminal portion of angiotensin I sequence as an inhibitor of human renin. Among compounds that we synthesized, benzyloxycarbonyl (Z)-Phe-His-Leucinal (compound V), Z-Pro-Phe-His-Leucinal (Compound IV) and Z-[3-(1'-naphthyl)Ala]-His-Leucinal (compound VII) markedly inhibited human renin ($IC_{50}$, $7.5 \times 10^{-7}$, $3.2 \times 10^{-7}$ and $8.0 \times 10^{-8}$ mol/l, respectively). Compound VII was shown to be noncompetitive ($K_i = 2.4 \times 10^{-7}$ mol/l). It did not inhibit either cathepsin D or pepsin. Compound V had slight or no inhibitory effect at the concentration of $10^{-5}$ mol/l on six animal renins except for monkey and rabbit renins. Results obtained show that these aldehyde compounds are highly selective and species specific inhibitors for human and monkey renins.

Renin (EC 3.4.99.19) catalyzes hydrolytic release of angiotensin I (ANG I) from the N-terminal end of angiotensinogen. ANG I is subsequently converted to the potent pressor peptide ANG II by angiotensin converting enzyme (ACE)(EC 3.4.15.1). Previously we reported that the ester of tetrapeptide in the sequence (Leu-Leu-Val-Tyr) of horse angiotensinogen (1) acted as an inhibitor of rabbit renin *in vitro* and *in vivo* (2,3). After our reports, various renin inhibitors were synthesized based on the N-terminal sequence of horse angiotensinogen (4-7). Human renin cleaves angiotensinogen from other mammals, but only primate renin hydrolyzes human angiotensinogen. Recently the N-terminal sequence of human angiotensinogen was elucidated (8). Szelke et al. (9) have prepared potent inhibitors of human renin by reducing the $Leu^{10}$-$Leu^{11}$ scissile bond in the human octapeptide $His^6$-$Pro^7$-$Phe^8$-$His^9$-$Leu^{10}$-$Val^{11}$-$Ile^{12}$-$His^{13}$.

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From the results of Szelke et al. (9) that the reduction of the scissile Leu-Val bond increased the inhibitory potency against human renin, and the findings of Umezawa and Aoyagi (10) that the C-terminal aldehyde group was critical for several potent protease inhibitors of microbial origin, we postulated that small peptides in ANG I sequence with leucinal at C-terminus might inhibit renin, if the peptide analogues could potentially interact with subsites in the catalytic center of renin. We synthesized derivatives of R-His-Leucinal and tested their inhibitory effect on human renin.

## MATERIALS AND METHODS

Human kidney renin was prepared by the method described previously (11). Human angiotensinogen was prepared by anti-human angiotensinogen antibody-Affi-Gel 10 affinity column chromatography and gel filtration on Ultrogel AcA 44 (12). Monkey, pig, goat, dog, rabbit and rat renins which were prepared by the method described previously (13) were further purified by gel filtration on Ultrogel AcA 44. Sheep, dog, rabbit and rat angiotensinogens from bilaterally nephrectomized plasma were prepared according to the method of Sen et al. (14). Pig angiotensinogen, bovine cathepsin D and porcine pepsin were purchased from Sigma (St. Louis, MO). Samples of purified human kidney ACE and aminopeptidase N (EC 3.4.11.2) were described previously (15,16).

Details of the syntheses of derivatives of R-His-Leucinal will be reported elsewhere.

Renin and angiotensinogen reactions were performed in the presence and absence of the compound in 0.1 mol/l phosphate buffer, pH 7.3 containing angiotensinase inhibitors at 37 ℃ for 10 min (17). ANG I generated was measured by radioimmunoassay of ANG I (13). Cathepsin D and pepsin were assayed using hemoglobin as substrate (18).

## RESULTS AND DISCUSSION

Derivatives of R-His-Leucinal which were tested in this study are shown in Table 1. Compounds III-VIII did not cross-react with ANG I antibody at the concentration of $10^{-5}$ mol/l. The minimal sequence required for inhibition of renin was tripeptide aldehyde Phe-His-Leucinal. The Dixon plot (19) showed that compound VII inhibited human renin noncompetitively as shown in Fig. 1. Ki value for human renin with human angiotensinogen was $2.4 \times 10^{-7}$ mol/l and that with sheep angiotensinogen was $6.8 \times 10^{-8}$ mol/l at pH 7.3. A slight decrease in inhibitory potency of human renin with human angiotensinogen may be due to different optimal pH in reactions of human renin-human angiotensinogen (pH 5.5-6.0) and human renin-sheep angiotensinogen (pH 7.0-7.5). When the aldehyde group of leucinal at the C-terminus of compound VII was substituted with alcohol group, the inhibitory potency ($3.5 \times 10^{-6}$ mol/l) of the

Table 1 Sequence of Peptide Aldehydes and Their Inhibitory Potencies for Human Renin

| Compound No. | Compound | $IC_{50}$, mol/l |
|---|---|---|
| I | Z-Tyr-Ile-His-Pro-Phe-His-Leucinal | no inhibition* |
| II | Z-Ile-His-Pro-Phe-His-Leucinal | no inhibition* |
| III | Z-His-Pro-Phe-His-Leucinal | $5 \times 10^{-5}$ |
| IV | Z-Pro-Phe-His-Leucinal | $3.2 \times 10^{-7}$ |
| V | Z-Phe-His-Leucinal | $7.5 \times 10^{-7}$ |
| VI | Z-His-Leucinal | $5 \times 10^{-4}$ |
| VII | Z-[3-(1'-naphthyl)Ala]-His-Leucinal | $8.0 \times 10^{-8}$ |
| VIII | Z-[3-(1'-naphthyl)Ala]-His-Leucinol | $5.6 \times 10^{-6}$ |

Z, benzyloxycarbonyl; *, at the concentration of $5 \times 10^{-4}$ mol/l

The test compounds were dissolved in 60 % ethanol. Human renin activity in the presence and absence of each compound was measured using sheep angiotensinogen. The total volume of 1 ml assay mixture contained 0.1 mol/l phosphate buffer, pH 7.3, human renin (0.5 ng ANG I/min), sheep angiotensinogen (200 ng ANG I equivalents), 7 different concentrations of each compound, 6 % ethanol and angiotensinase inhibitors (10 mmol/l EDTA and 3.4 mmol/l 8-hydroxyquinoline). The reaction was carried out for 10 min at 37 ℃ and then stopped by placing the tube in a boiling water bath for 5 min. After centrifugation, the supernatant (50-100 µl) was used for assay of ANG I. Values were the mean of 3 or 4 experiments.

compound decreased with change of inhibition type from noncompetitive to competitive (Fig. 1).

We tested the ability of compound VII to inhibit acid proteases, cathepsin D and pepsin at concentrations from $10^{-4}$ to $10^{-6}$ mol/l. The compound did not inhibit either cathepsin D or pepsin even at the concentration of $10^{-4}$ mol/l. Compound IV and V at $10^{-5}$ mol/l had practically no inhibitory effect

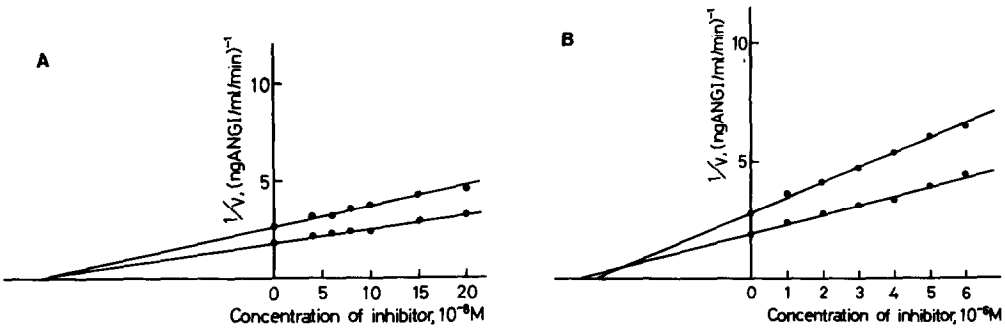


Figure 1. The Dixon plot of the inhibition of human renin by compound VII and VIII for two concentrations of human angiotensinogen ($S_1$ = 200 ng ANG I equivalents; $S_2$ = 100 ng ANG I equivalents). Human renin activity was 0.5 ng ANG I/ml of assay volume/min at pH 7.3 and 37 ℃. The reaction was carried out for 10 min at pH 7.3 and 37 ℃ under the condition described in the Table 1.
A: compound VII, Z-[3-(1'-naphthyl)Ala]-His-Leucinal
B: compound VIII, Z-[3-(1'-naphthyl)Ala]-His-Leucinol

Table 2 Species Specificity of Renin Inhibitor (Compound V)

| Renin | Angiotensinogen | Inhibitory % ($10^{-5}$ mol/l) |
|---|---|---|
| Human | Sheep | 85 |
| Monkey | Sheep | 87 |
| Pig | Pig | 11 |
| Goat | Sheep | 19 |
| Dog | Dog | 4 |
| Rabbit | Rabbit | 70 |
| Rat | Rat | 4 |

All renin-angiotensinogen reactions were carried out for 10 min at 37 °C as described in the Table 1. The generation of ANG I in the presence of inhibitor was expressed as a percent of that without inhibitor. Activities of renins tested were all about 0.4-0.5 ng ANG I/ml of incubation mixture/min with about 200 ng ANG I equivalents of the corresponding angiotensinogen. Each value was the mean of 3 experiments.

on human ACE and aminopeptidase N. Thus, the peptide aldehydes seem to be highly selective for renin.

The inhibitory effect of compound V on animal renins was studied (Table 2). It had slight or no inhibitory effect at the concentration of $10^{-5}$ mol/l on all animal renins except for monkey and rabbit renins. These results suggest that peptide aldehydes are highly species specific for human renin.

We have reported that compound I inhibits hog renin activity more than 50% at the concentration of $7.7 \times 10^{-6}$ mol/l with acetyltetradecapeptide as substrate (20). The inhibitory effect of compound V on hog renin was very weak as shown in Table 2. The elongation of peptide chain progressively increased the inhibitory effect on hog renin (20). However, human renin was not inhibited by compound I or II at the concentration of $5 \times 10^{-4}$ mol/l. Boger et al. (21) have reported potent inhibitors of renin in which statine is incorporated into horse octapeptide $His^{6}$-$Pro^{7}$-$Phe^{8}$-$His^{9}$-$Leu^{10}$-$Leu^{11}$-$Val^{12}$-$Tyr^{13}$. Among them the compound, Iva-His-Pro-Phe-His-Sta-Ile-Phe, is the most potent inhibitor of human renin. But it also inhibits acid proteases such as cathepsin D and pepsin considerably. The compound VII did not inhibit either cathepsin D or pepsin. Compounds V and VII are potent and specific inhibitors of human renin. The inhibition of renin *in vivo* using compound VII or VIII is currently in progress.

Renin has a major role in the maintenance of certain types of hypertension (22). The development of ACE inhibitor captopril as an antihypertensive drug made a great progress in the control of not only renin-dependent hypertension but also essential hypertension (23-25). This again evoked the interest and importance of the development of potent renin inhibitors. The design of aldehyde of short peptide chains may provide a possibility of the development of an orally active renin inhibitor available for medical treatment.

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