Hemodynamic and Humoral Effects of the New Renin Inhibitor Enalkiren in Normal Humans

Alain Delabays, Jürg Nussberger, Marinette Porchet, Bernard Waeber, Patricia Hoyos, Robert Boger, Harriet Glassman, Hollis D. Kleinert, Robert Luther, and Hans R. Brunner

The effect of the renin inhibitor enalkiren (Abbott-64662) was evaluated in eight normal volunteer subjects on a standardized sodium diet (100 mmol/day) by measurement of various components of the renin-angiotensin system and drug levels in plasma. On day 1, vehicle and doses of 0.001, 0.003, and 0.01 mg/kg i.v. were administered within 2 minutes at 90-minute intervals. On day 2, vehicle and doses of 0.01, 0.03, and 0.1 mg/kg i.v. were given. With the higher doses, blood pressure tended to decrease slightly with no change in heart rate. Plasma renin activity and plasma angiotensin-(1-8)octapeptide (angiotensin II) fell markedly in a dose-dependent manner. Inhibition of plasma renin activity was maximal 5 minutes after administration of the drug and persisted 90 minutes after the doses of 0.03 and 0.1 mg/kg. Not surprisingly, there was a close correlation between plasma renin activity and plasma angiotensin II levels (r=0.81, n=28, p<0.001). In contrast, active and total renin measured directly by monoclonal antibodies rose in dose-related fashion in response to renin inhibition. Pharmacokinetic parameters were calculated using the plasma drug concentrations obtained up to 6 hours after the 0.1 mg/kg dose. By means of a two-compartment model, plasma mean half-life of the drug was estimated at 1.60±0.43 hours. (Hypertension 1989;13:941–947)

Over the last decade, inhibition of the renin-angiotensin system by converting enzyme inhibition has proved very effective for the treatment of hypertension and congestive heart failure. Converting enzyme inhibitors block the generation of angiotensin II, a potent pressor hormone. Because converting enzyme is identical to kininase II, it also contributes to the inactivation of the vasodilator hormone bradykinin. Accordingly, converting enzyme inhibition does not provide a totally specific means of blocking the renin-angiotensin system. Furthermore, the disappearance of angiotensin II from the plasma triggers counterregulatory mechanisms that tend to limit the efficacy of angiotensin converting enzyme (ACE) inhibition. Thus, renin secretion is stimulated and, as a consequence, plasma angiotensin I is elevated. Even in the presence of pronounced ACE inhibition, some angiotensin II may still be generated.

More specific tools for blocking of the renin-angiotensin system would be useful. Renin inhibitors have the theoretical advantage of specificity since renin has no known substrate other than angiotensinogen. So far, at least experimentally, renin has been blocked by means of various approaches: specific renin antibodies, phospholipids, peptides related to the renin prosegment, pepstatin and its derivatives, and analogues of the renin substrate. The latter are derived from the minimal sequence of angiotensinogen reacting with renin. Actual inhibitors of this class contain a reduced bond between two amino acids at the cleavage site of renin. The inhibitors seem to act by mimicking the transition state of the substrate formed during hydrolysis. Such compounds have been reported to effectively block plasma renin activity in primates and in humans.

The aim of the present study was to investigate in normal volunteers the effect of the new renin inhibitor enalkiren (Abbott-64662) on circulating renin and angiotensin II after intravenous administration of five different doses on 2 consecutive days. In addition, plasma drug levels were determined for calculation of preliminary estimates of pharmacokinetic parameters.
Subjects and Methods

Eight healthy volunteers, aged 20–23, participated in the study. All of the subjects were fully informed of the goals and potential risks of the investigation and gave written consent. The protocol was approved by the institutional ethical committee.

The volunteers received dietary instructions that specified a sodium intake approximating 100 mmol/day during the week before the study. Compliance was verified by measurement of sodium excretion by urine collection obtained during the 24 hours before the experiment. On this day the subjects were also familiarized with blood pressure measurements and cardiac monitoring procedures. They then came to the clinical study facility at 7:00 AM after an overnight fast and were placed in supine position. The ECG was monitored with an HP Model 7380 (Hewlett-Packard Co., Andover, Massachusetts). Blood pressure and heart rate were determined every 5 minutes by an automatic blood pressure measuring device (Dinamap SXP, Critikon, Tampa, Florida). Blood was drawn for determination of plasma renin activity after 60 minutes of rest with the subjects in supine position and after 5 minutes with the subjects standing.

On the first study day, all volunteers were placed in supine position and monitored in an identical fashion. Intravenous cannulas were inserted in both arms, one for infusion of the drug and one for blood sampling. After a stabilization period of 1 hour, vehicle (NaCl 8.78 g/l, acetic acid 0.182 g/l) and the renin inhibitor enalkiren (doses of 0.001, 0.003, and 0.01 mg/kg) were injected intravenously at 90-minute intervals as rapid infusions 2 minutes in duration. Each infusion, which had a volume of 26 ml, was injected by an automatic pump (Doltron PIM 717, Doltron AG, Uster, Switzerland). Blood pressure and heart rate were measured every 5 minutes until 4 hours after the last dose. Blood samples for determination of plasma renin activity and active and total renin were taken 5 minutes before and 5, 15, 30, 45, and 90 minutes after each infusion. Plasma drug levels were quantitated in the same samples. On the second study day, the same procedure was repeated with successive intravenous infusions of vehicle and enalkiren at 0.01, 0.03, and 0.1 mg/kg. Additional blood samples were collected 120, 180, and 360 minutes after the highest dose. On both study days, plasma angiotensin II was measured before and 5 minutes after the last and highest dose of active drug.

Blood samples were processed immediately at 4°C, and the plasma samples were stored at −70°C. Plasma renin activity was measured by radioimmunoassay quantitating angiotensin I generated during a 30-minute incubation and trapped by antibody as first described by Poulsen and Jorgensen and adapted for our laboratory. Active and total renin was determined by a direct radioimmunoassay using monoclonal antibodies. Blood samples for the determination of circulating angiotensin II were collected in prechilled tubes containing a renin inhibitor (CGP-29287, CIBA-GEIGY, Basel, Switzerland) and immediately centrifuged at 4°C; the plasma was quickly frozen in liquid nitrogen. Angiotensin-(1–8)octapeptide was then measured with a radioimmunoassay after a prior separation of other angiotensins by high-performance liquid chromatography (HPLC). No cross-reaction of enalkiren was observed with any antibody used when the renin inhibitor was added to human plasma in a 1.2×10^5-fold molar excess compared with the endogenous hormone to be measured. Plasma levels of enalkiren were determined by reversed-phase HPLC using ultraviolet detection. The time-concentration profile of the drug was best described by a two-compartment model using the NONLIN-84 nonlinear regression software package.

The renin inhibitor enalkiren (A-64662) was synthesized by Abbott Laboratories, Abbott Park, Illinois. It is basically a transition-state peptide analogue of angiotensinogen [N-(3-amino-3-methyl-1-oxobutyl)-4-methoxy-L-phenylalanine]-N'-(15S,2R,3S)-1-cyclohexylmethy l-2,3-dihydroxy-5-methylhexyl]-L-histidinamide. The compound was diluted by vehicle to a concentration that provided the intended dose per weight in a final volume of 26 ml.

The data were evaluated based on a two-way analysis of variance (ANOVA) for comparison of the effect of the vehicle and that of the different doses of the drug. When overall statistical significance was achieved (p<0.05), the least significant difference method was used for comparison of individual mean values. Regression analysis was performed when indicated. A paired t test was used for comparison of plasma angiotensin II values before and after administration of the drug.

Results

The mean urinary sodium excretion of the volunteers during the day preceding the study was 97.2±23 mmol/24 hr (mean±SEM). There was no change in plasma renin activity with the subjects in supine position and after 5 minutes of standing on the day before (1.34±0.3 and 1.38±0.28 ng angiotensin I [Ang I]/[ml • hr], respectively) as well as on the day after the study (1.06±0.2 and 1.2±0.26 ng Ang I/ [ml • hr], respectively).

Figure 1 illustrates the effect of the renin inhibitor enalkiren on blood pressure, heart rate, and plasma renin activity. The results of the first study day are depicted in the upper panel, and those of the second study day are depicted in the lower panel. There was no consistent change in blood pressure or heart rate at any time during the study. Nevertheless, within 15 minutes after the highest dose, blood pressure fell slightly from 111/61±3.7/2.1 to 107/52±2.7/2.5.

Plasma renin activity was reduced in dose-related manner by enalkiren. After the two vehicle infusions, plasma renin activity varied between 0.93±
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Vehicle 0.001 mg/kg 0.003 mg/kg 0.01 mg/kg

FIGURE 1. Time course of changes in blood pressure, heart rate, and plasma renin activity (PRA) after bolus intravenous injection of the renin inhibitor enalkiren (Abbott-64662) on study day 1 (upper panel) and study day 2 (lower panel). Values are expressed as mean±SEM. Ang I, angiotensin I.

0.25 and 1.01±0.24 ng Ang I/(ml • hr). Peak effect of the drug was observed 5 minutes after infusion. After the 0.1-mg/kg dose, plasma renin activity had fallen to 0.09±0.04 ng Ang I/(ml • hr). With the lowest doses, plasma renin activity tended to return to its predrug value 90 minutes after administration. In contrast, with doses of 0.03 and 0.1 mg/kg, pronounced inhibition was still present after 90 minutes. The relation between the dose administered and plasma renin activity measured 5 minutes after drug administration is illustrated in Figure 2.

Figure 3 depicts individual and mean plasma angiotensin II before and 5 minutes after doses of 0.01 and 0.1 mg/kg enalkiren. The lower dose decreased plasma angiotensin II from 6.71±1.69 to 3.81±1.23 femtomole/ml (p<0.05) and the higher dose, from 5.0±1.04 to 0.62±0.15 fmol/ml (p<0.001). (For Figure 3 the two control values were averaged.) Plasma angiotensin II and renin activity determined at the same time showed a close correlation (r=0.81, n=28, p<0.001).

The changes in active and total renin determined by monoclonal antibodies are illustrated in Figure 4. Inactive renin is calculated by subtraction of active renin from total renin. Active renin tended to rise slowly even with the lowest doses. A pronounced increase from 31±6 to 48±10 pg/ml and from 43±7.5 to 66±15 pg/ml (mean±SEM) was seen with doses of 0.03 and 0.1 mg/kg, respectively, 30 minutes after administration of the drug. This increase began 15 minutes after the injection of the drug. The relation between the dose administered and active renin activity measured 30 minutes after drug administration is illustrated in Figure 2.

FIGURE 2. Mean dose–response curve of plasma renin activity 5 minutes after bolus intravenous injection of the renin inhibitor enalkiren (Abbott-64662). Values are expressed as mean±SEM. Ang I, angiotensin I.
Dose of renin inhibitor Abbott - 64662 mg/kg

FIGURE 3. Individual (○) and mean (●) ± SEM values of plasma angiotensin-(1–8) octapeptide before and 5 minutes after bolus intravenous injection of 0.01 and 0.1 mg/kg of enalkiren (Abbott-64662). Each zero point represents the mean of two determinations.

Discussion

These results demonstrate that infusion of the renin inhibitor enalkiren produced a dose-dependent reduction in plasma renin activity of normal subjects. Increasing doses enhanced both peak inhibition and duration of the decrease in plasma renin

0.1 mg/kg are illustrated in Figure 5. The initial disappearance of the drug, corresponding mostly to the distribution phase, was rapid and parallel for each dose. The pharmacokinetics of enalkiren were determined only from plasma levels obtained at the highest dose, for which plasma samples were collected over a 6-hour period. The analysis gave a mean half-life for the rapid distribution phase of 15±6 minutes. The mean elimination half-life, calculated from the terminal portion of the curve, was 1.60±0.43 hours. Estimates for the relative volume of distribution of the central compartment and area under the curve values averaged 0.044±0.003 l/kg and 2,491±609 (ng · hr)/ml, respectively.

Enalkiren was well tolerated by all subjects. One volunteer had to be withdrawn from the study because of a malaise immediately after injection of the vehicle and the lowest dose of the drug. Injection of saline was well tolerated. We attributed the discomfort to the presence of acetic acid in the vehicle of the drug. Cutaneous hypersensitivity to this substance could not be demonstrated.
activity. Angiotensin II levels, measured before and after the 0.01 and 0.1 mg/kg doses, also were greatly reduced in dose-dependent fashion. After the highest dose, plasma angiotensin II fell to very low levels. In these salt-replete normal volunteers, only a slight and transient reduction in blood pressure was observed after the highest dose of the renin inhibitor with no change in heart rate.

Why test a renin inhibitor in normotensive volunteers? These agents are clearly designed to antagonize the interaction of renin with its substrate to reduce the generation of angiotensin I and ultimately of angiotensin II. Since the drug was designed for this purpose, it seems logical to test this action when the drug is first administered to humans. Normotensive volunteers provide a relatively homogeneous study population, in clear contrast with hypertensive patients. By use of appropriate methodology, it is then possible to demonstrate the decrease in plasma renin activity and, much more importantly, the dose-dependent reduction in angiotensin II levels.

Despite the decrease in plasma angiotensin II, blood pressure was not reduced. This is not surprising at all since in our hands, salt-replete normal volunteers have not shown consistent reduction in blood pressure after administration of an ACE inhibitor. No fall in blood pressure was observed in normal volunteers after intravenous administration of captopril in doses that completely suppressed plasma angiotensin II levels. It should be kept in mind that use of blood pressure as an end point for testing the efficacy of a drug that interferes specifically with one hypertensive mechanism is only valid if it is established beyond any doubt that the blood pressure under the circumstances prevailing during the testing is really dependent on this pressor mechanism. For instance, there would probably be little disagreement that the renin-angiotensin system does not play a predominant role in all hypertensive patients, and there are clearly some patients in whom complete blockade of the system would not be expected to reduce blood pressure. Renin inhibitors can be expected to reduce plasma angiotensin II in every subject to whom they are administered. However, they would not be expected to reduce blood pressure in every normotensive or hypertensive patient. Logically, therefore, one would not expect to find a correlation between the drop in angiotensin II and the blood pressure reduction induced by a renin inhibitor. The measurement of blood pressure tests the hypothesis of whether blood pressure maintenance of the subject to whom the renin inhibitor was given is dependent on angiotensin II, and not whether the renin inhibitor is pharmacologically effective, that is, whether it reduces angiotensin II.

During recent years, there has been accumulating evidence that renin is synthesized not only in the kidney but also in the brain, in the heart, and in vascular wall, and that complete renin-angiotensin systems may exist in these various tissues. Based on these findings, it could be argued that the renin inhibitor reduces circulating angiotensin II but not angiotensin II levels in tissues, and that this is why blood pressure did not fall in our normotensive subjects. Similar arguments have been put forward in the context of the antihypertensive effect of ACE inhibitors. In a clinical setting, it is very difficult to either confirm or disprove this hypothesis since measurements in tissue, independently of the methodological difficulties, are in most instances not feasible. More experimental studies are needed on the accurate simultaneous measurement of angiotensin II in plasma and in these tissues; however, many methodological problems remain to be solved for successful completion of such experiments.

The first group to report the effects of a renin inhibitor (H142) in humans was Webb and co-workers. These investigators reported results sim-
ilar to ours, that is, a fall in plasma renin activity, angiotensin I, and angiotensin II in the face of a rise in plasma renin concentration. They observed no change in systolic blood pressure but did detect a decrease in diastolic pressure. The increase in plasma renin concentration was thought to result from a lack of feedback inhibition by circulating angiotensin II on renin secretion. In the present study, we observed a dose-dependent increase in active and total renin that could be measured by direct radio-immunoassays using three types of monoclonal antibodies. It is interesting that immediately after renin inhibition, mainly active renin also increased in a manner similar to that in acute ACE inhibition. Only after repeated administration of an ACE inhibitor did inactive plasma renin start to rise.

The measurement of plasma renin activity is often used exclusively to evaluate the effect of renin inhibitors. Frequently this measurement provides convincing results, but this is not necessarily the case. It should be kept in mind that plasma renin activity is not measured in vivo. The determination introduces several confounding factors, such as changes in pH and presence of inhibitors of angiotensinases and converting enzyme. Substrate concentrations and incubation time may also influence results. If plasma renin activity is used as the sole end point measured, and particularly if the results obtained do not correspond to the expected effect, it is very important to validate the method by relating its results to measured plasma angiotensin levels. This validation should be repeated for each new renin inhibiting agent because, depending on the affinity of the compound and small changes in the assay procedure, clear dissociations between the results of plasma angiotensin and plasma renin activity might occur with one agent but not another.

Of note is the duration of plasma renin activity suppression achieved with enalkiren. Although Webb and coworkers reported a decrease in angiotensin I and angiotensin II levels during the 30-minute infusion of the renin inhibitor H142 but prompt return to baseline values after its discontinuation, in our study plasma renin activity values remained suppressed approximately 60% below baseline values for up to 6 hours after drug administration. Extended duration of action has also been observed in animal studies. The half-life for enalkiren, calculated at 1.6 hours in the present study, is actually even longer than anticipated from previous animal data. Obviously, the extended duration of biologic activity and the relatively long half-life are encouraging for the potential development of a renin inhibitor for therapeutic use.

Taken together, the results of this study clearly demonstrate that, when administered parenterally to normal volunteers, enalkiren exerts the effect for which it was designed. Thus, it markedly reduces plasma renin activity and, most importantly, circulating angiotensin II levels. Renin inhibitors have been shown to reduce blood pressure of hypertensive animals in which blood pressure was known to be angiotensin II–dependent. Accordingly, it can be expected that this renin inhibitor will also reduce blood pressure of patients with angiotensin II–dependent hypertension.

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References


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