Hemodynamic and Biochemical Consequences of Renin Inhibition by Infusion of CGP 38560A in Normal Volunteers

Jürg Nussberger, Alain Delabays, Marc De Gasparo, Frédéric Cumin, Bernard Waeber, Hans R. Brunner, and Joël Ménard

Hemodynamic and biochemical effects of the new renin inhibitor CGP 38560A (molecular weight 826) were tested in 15 healthy volunteers after a single-blind, randomized, placebo-controlled protocol. At a 2-week interval, groups of five subjects received a 30-minute infusion of either 5% dextrose or CGP 38560A 50, 125, or 250 μg/kg. Blood pressure, heart rate, plasma renin activity, active and total renin, angiotensin-(1-8)octapeptide (angiotensin II), and aldosterone were sequentially measured up to 3 hours from the onset of the infusion. There was no consistent change in blood pressure or heart rate. Plasma renin activity and angiotensin II decreased dose dependently, and peak suppression was observed at the end of the infusion of CGP 38560A and after the 250 μg/kg dose. Plasma renin activity fell from 1.0±0.19 (mean±SEM) to less than 0.05 ng/ml/hr in all five subjects (p<0.001), and angiotensin II fell from 7.7±1.2 to 2.6±0.9 femtomole/ml (p<0.01). Active renin rose fourfold from 24±1.9 to 98±14 pg/ml (p<0.001) at the end of the infusion of the high dose. Plasma angiotensin II returned toward its initial values much faster than plasma renin activity and active renin. In conclusion, CGP 38560A was well tolerated. It induced a dose-dependent decrease in angiotensin II and plasma renin activity and a long-lasting and dose-dependent rise in active renin. The doses used did not reduce plasma angiotensin II maximally despite reduction of plasma renin activity to unmeasurable levels. Thus, the measurement of plasma renin activity cannot replace that of plasma angiotensin II to evaluate the efficacy of renin inhibitors.

The success of angiotensin converting enzyme inhibitors in the therapy of hypertension and heart failure is mainly attributed to decreased production of angiotensin-(1-8)octapeptide (Ang II). The lack of specificity of converting enzyme, which also modifies the metabolism of at least bradykinin and substance P, might play a role in causing side effects such as cough or angioneurotic edema. Drugs that inhibit Ang II generation without modifying the metabolism of bradykinin and other peptides may lack untoward effects of converting enzyme inhibitors. This goal could be achieved by preventing the generation of angiotensin I (Ang I) instead of blocking its conversion to Ang II. Since renin generates Ang I from angiotensinogen, it was logical to attempt inhibition of this first, specific, and rate-limiting reaction of the renin-angiotensin system. The purpose of the present study was to evaluate the new renin inhibitor CGP 38560A. By comparison with the two renin inhibitors that have been administered to human beings, the renin inhibitory peptide\(^1\) and the H-142,\(^2\) CGP 38560 has a molecular weight (mol wt) of 826 instead of 1,319 and 1,210, respectively. CGP 38560A seems more potent in vitro since its IC\(_{50}\) was calculated at 0.7 nM compared with 2 μM for the renin inhibitory peptide and 10 nM for the H-142. The effects of the CGP 38560A on blood pressure and the main biochemical elements of the plasma renin-angiotensin system were tested in healthy human volunteers after mild sodium depletion.

Subjects and Methods

Fifteen healthy male volunteers aged 20–29 years received a 30-minute infusion of CGP 38560A (50, 125, or 250 μg/kg) and 5% dextrose (vehicle) in a randomized, single-blind order at a 2-week interval. CGP 38560A (CIBA-GEIGY Ltd, Basel, Switzer-
land) is a potent (IC\textsubscript{50} 0.7 nM) low molecular weight (mol wt 826) renin inhibitor that consists of the methanesulfonate of N-(2(R)-benzyl-3-test-butyl-sulfonyl-propionyl-)-His-Cha-Val-n-butylamide.\textsuperscript{3} The study was in agreement with the Declaration of Helsinki\textsuperscript{4} and was accepted by the hospital's ethics committee. Informed consent was obtained from each participating subject before entering the study. The subjects remained on an unrestricted diet throughout the study and were given 40 mg furosemide p.o. 15 hours before infusion. Urine was collected 24 hours before and 14 hours after diuretic administration. Additional collections of urine were obtained on the day of the study (from 8:00 AM to 12 noon) and finally during the following 20 hours. Food intake was stopped 15 hours before the onset of infusion; liquid intake was stopped 11 hours before infusion.

On the day of the study, the fasting subjects came to the hospital ward at 8:00 AM. They remained in supine position throughout the study. Indwelling cannulas (Venflon, Viggo AG, Helsingborg, Sweden) were inserted into veins of the right and left arm for infusion and blood sampling. CGP 38560A was dissolved in 5% dextrose (vehicle). The solution was sterilized by passing it through a filter (Millipore, Molsheim, France) of 0.45 \textmu m pore size. Beginning at 9:00 AM, a total of 11 ml CGP 38560A or vehicle was infused during 30 minutes at a constant rate (Doltron pump PIM 717, Doltron AG, Uster, Switzerland). From 8:00 AM to 12 noon heart rate and blood pressure (BP monitor 3100, EME, Brighton, England) were measured at 5-minute intervals, and the electrocardiogram (ECG) was continuously monitored (HP 7380, Hewlett-Packard Co., Andover, Massachusetts). Blood samples were obtained before and 15, 30, 60, 120, and 180 minutes after onset of the infusion for measurement of plasma renin activity (PRA),\textsuperscript{5,6} Ang II,\textsuperscript{7} aldosterone,\textsuperscript{8} plasma active\textsuperscript{9} and total renin concentration,\textsuperscript{10} and drug levels. Determinations of the renin inhibitor concentration in plasma was performed by a radio-receptor assay. Semipurified human renin (specific activity 150–200 Goldblatt units/mg protein) was incubated with \textsuperscript{[3H]}CGP 38560 in 0.1 M trisacetate buffer, pH 5.7, in the presence of various concentrations of CGP 38560 for 2 hours at room temperature. The bound and the free fractions were separated by the dextran-coated charcoal method. The amount of radioactive renin inhibitor complex was inversely proportional to the amount of CGP 38560, and a standard curve was constructed from which the concentration of unknown plasma could be read. The threshold for the detection of the renin inhibitor in plasma was 0.9 nmol/l. The assay measures the free base (CGP 38560) of the renin inhibitor salt (CGP 38560A) and any metabolite able to bind to human renin. The intra-assay and interassay variations were 12.5±1.3% (n=10) and 20.0±2.1 (n=10), respectively. Results are expressed as nanomole equivalent per liter.

Results are mean±SEM. Significance of differences between means was evaluated by two-way analysis of variance. Appropriate means were compared by Fisher's least significant difference test. Values of p<0.05 were considered statistically significant.

**Results**

**Blood Pressure, Heart Rate, and Diuresis**

The infusion of CGP 38560A was well tolerated by all subjects, and no untoward effects or ECG abnormalities were observed. Heart rate and systolic and diastolic blood pressure did not change significantly throughout the study. Furosemide increased diuresis from 838±67 to 1,833±91 \textmu l/min (p<0.001). Urinary sodium excretion rose from 88±6 to 215±8 \textmu mol/min (p<0.001). Diuresis and

**FIGURE 1.** Plots showing effect of a 30-minute intravenous infusion of renin inhibitor CGP 38560A on plasma renin activity (PRA) and plasma angiotensin-(1–8)octapeptide in slightly sodium-depleted healthy volunteers. Both variables decrease dose dependently during infusion and return to baseline after infusion.
natriuresis were not modified during and after the infusion of the renin inhibitor.

**Renin Activity, Angiotensin II, and Aldosterone**

The dose-dependent effects of CGP 38560A infusion on PRA and Ang II are shown in Figure 1. Both variables remained unchanged during infusion of vehicle. In contrast, PRA decreased ($p<0.001$) 15 minutes after starting CGP 38560A infusion (from $1.44±0.11$ to $0.28±0.15$ ng/ml/hr with $50 \mu$g/kg CGP 38560A, from $1.02±0.26$ to $0.12±0.04$ ng/ml/hr with $125 \mu$g/kg CGP 38560A, and from $1.04±0.19$ to $0.01±0.01$ ng/ml/hr in all subjects receiving $250 \mu$g/kg CGP 38560A). At the end of the infusion, PRA was further suppressed by infusion of CGP 38560A at the low dose to $0.16±0.04$ ng/ml/hr. PRA remained unchanged with the two higher doses at $0.14±0.04$ and less than $0.05±0$ ng/ml/hr, respectively. During the recovery period, PRA gradually returned to pretreatment values. Ninety minutes after infusion, PRA was still significantly ($p<0.05$) decreased at $0.57±0.23$ ng/ml/hr after infusion of $250 \mu$g/kg CGP 38560A only.

Ang II was decreased 15 minutes after starting the infusion of CGP 38560A at the low dose from $7.4±1.8$ to $3.6±0.7$ fmol/ml ($p<0.01$ vs. vehicle-control at $7.2±0.8$ fmol/ml), at the intermediate dose from $8.2±1.5$ to $2.3±0.7$ fmol/ml ($p<0.01$ vs. baseline and $p<0.001$ vs. vehicle at $7.5±1.3$ fmol/ml), and at the high dose from $7.7±1.2$ to $1.9±0.4$ fmol/ml ($p<0.001$ vs. baseline and vehicle at $9.8±1.1$ fmol/ml). At the end of the CGP 38560A infusion, Ang II remained low. However, at the $50 \mu$g/kg dose, the decrease in Ang II was no longer statistically significant ($5.0±1.9$ fmol/ml, $p>0.05$). During the recovery period, Ang II levels returned to pretreatment values. Thirty minutes after the end of infusion, Ang II was still significantly reduced ($5.1±0.9$ fmol/ml, $p<0.01$ vs. vehicle at $11.2±0.8$ fmol/ml) when CGP 38560A had been infused at the $250 \mu$g/kg dose. Ang II levels were correlated with the renin inhibitor. At the end of the infusion, Ang II was no longer statistically significant ($p<0.05$) if values after CGP 38560A infusion were used ($r=0.76$, $n=180$, $p<0.001$; $y=4.2x±3.2$), and this correlation remained unchanged only if values after CGP 38560A infusion were used ($r=0.73$, $n=90$, $p<0.001$; $y=4.4x±2.8$).

Although the levels of PRA and plasma Ang II seem to change in parallel, Ang II returned to its initial levels at a rate faster than PRA. Thirty minutes after the end of the CGP 38560A infusion, plasma Ang II was no longer significantly different from its initial levels ($0\%$, $30\%$, and $34\%$ decrease), at a time when PRA was still inhibited by $54\%$, $77\%$, and $82\%$, respectively, by the $0.05$, $0.125$, and $0.250$ mg/kg doses of CGP 38560A (Table 1).

Plasma aldosterone concentrations (PA) are shown in Table 1. Mean PA remained unchanged after vehicle infusion ranging between $66.1±7.5$ and $74.9±9.6$ pg/ml. PA decreased after CGP 38560A infusion; the lowest PA were measured 30 minutes after the end of the $250 \mu$g/kg dose ($44.6±8.9$ pg/ml, $p<0.01$ vs. vehicle). At the same time, but after the
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Renin Inhibition by CGP 38560A in Humans  

TABLE 2. Plasma Concentration of Renin Inhibitor CGP 38560A During and After Its Intravenous Infusion Over 30 Minutes in Five Normal Volunteers

<table>
<thead>
<tr>
<th>Time after onset of infusion (min)</th>
<th>Dose 1 (50 µg/kg)</th>
<th>Dose 2 (125 µg/kg)</th>
<th>Dose 3 (250 µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>303±27</td>
<td>810±81</td>
<td>1,342±72</td>
</tr>
<tr>
<td>30</td>
<td>470±27</td>
<td>1,078±146</td>
<td>1,856±178</td>
</tr>
<tr>
<td>60</td>
<td>50±19</td>
<td>135±55</td>
<td>259±47</td>
</tr>
<tr>
<td>120</td>
<td>3.5±0.8</td>
<td>12±4.5</td>
<td>27±1.0</td>
</tr>
<tr>
<td>180</td>
<td>1.6±0.2</td>
<td>5.1±1.5</td>
<td>11±0.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM (nmol/l).

50 and 125 µg/kg dose, PA were 62.6±17.6 and 56.0±7.6 pg/ml, respectively.

Active, Inactive, and Total Renin

Plasma concentrations of active renin, total renin, and inactive renin are shown in Figure 2. Active renin increased in a dose-dependent form from 23.0±2.8 to 62.8±22.2 pg/ml (p<0.05), from 21.2±3.7 to 80.4±22.2 pg/ml (p<0.01), and from 24.2±1.9 to 97.8±14.1 pg/ml (p<0.001) in response to CGP 38560A infusions of 50, 125, and 250 µg/kg, respectively. Active renin levels remained high or reached peak values (125 µg/kg dose) 30 minutes after the end of the infusion and gradually decreased during the following 2 hours after the low CGP 38560A dose to 32.0±3.4 pg/ml (not different from baseline), after the intermediate dose to 44.6±10.8 pg/ml (p<0.05 above vehicle), and after the high dose to 50.4±3.0 pg/ml (p<0.05 above vehicle and baseline). Total plasma renin concentrations increased in parallel but without reaching statistical significance; during and after vehicle infusion, mean plasma levels of total renin remained remarkably constant with all values ranging between 140.4±9.4 and 141.7±9.4 pg/ml (n=15). Plasma inactive renin was calculated as the difference between total and active renin. In contrast to vehicle infusion, there was a tendency for a transient decrease in inactive renin concentration in response to CGP 38560A infusion, but none of these changes were statistically significant. For vehicle infusion, means ranged between 110.3±8.1 and 116.0±8.9 pg/ml; with infusion of 50, 125, and 250 µg/kg CGP 38560A, inactive renin levels decreased to 108.2±12.0 pg/ml, 104.0±10.8 pg/ml, and 98.6±24.6 pg/ml, respectively, before they returned to pretreatment values. For the two lower doses, this return occurred during infusion; for the highest dose, this return occurred during the recovery period.

Drug Levels

Plasma concentrations of CGP 38560A are summarized in Table 2. Peak levels were reached at the end of the infusion. They were 1,856±178, 1,078±146, and 470±27 nmol/l, for the high, intermediate, and low dose, respectively. The disappearance of CGP 38560A from plasma was extremely fast and was in accordance with the calculated value of 7.6 minutes for its rapid elimination phase.

Discussion

Several renin inhibitors have been studied in animals, but only two have been evaluated in humans. Since active renin represents the rate-limiting step in the production of Ang II, the potent effector hormone of the renin-angiotensin system, renin inhibitors are designed to counteract this renin activity and hence to limit the generation of Ang II. Investigation of the effect of renin inhibitors on the components of the human renin-angiotensin system must therefore be based on reliable monitoring of these variables, particularly on the measurement of Ang II concentrations, and technical aspects have to be considered when plasma renin activities are reported.

CGP 38560A did not change systolic or diastolic blood pressure in slightly salt-depleted volunteers. The absence of a fall in blood pressure seems at variance with the results obtained with H-142. In reality, the sodium depletion induced in the normal volunteers exposed to H-142 was more drastic than the depletion induced in our volunteers. The blood
pressure of our volunteers was therefore much less dependent on the renin-angiotensin system. In normal volunteers injected with captopril intravenously, we also observed no fall in blood pressure despite a dose-dependent fall in plasma Ang II to nondetectable levels.\textsuperscript{12} CGP 38560A was well tolerated by all volunteers, and no side effects were noticed in the presence of peak plasma levels of CGP 38560A, which were more than three orders of magnitude higher than the in vitro inhibition constant ($K_i$) value reported earlier.\textsuperscript{3} Urinary electrolyte excretion remained unchanged during and after the infusion of the renin inhibitor.

PRA decreased in dose-dependent fashion during CGP 38560A infusion and fell below the detection limit with the highest dose. It was no longer different from the initial value 90 minutes after the end of the infusion with the lower doses of the renin inhibitor, but it was reduced by 45% after the infusion of the highest dose. Whereas the initial values of PRA were not different from those usually measured in normal volunteers on a normal sodium diet, plasma Ang II was twofold higher as a consequence of the mild sodium depletion induced by furosemide, which already suggests that plasma Ang II is more sensitive than PRA in revealing the mild stimulation of the renin-angiotensin system. During CGP 38560A infusion, plasma Ang II concentrations fell rapidly in a dose-dependent manner but remained well above the lower limit of detection even with the highest dose. This finding contrasts with the results obtained in previous experiments; for example, after the intravenous administration of captopril to normal volunteers, plasma Ang II levels always fell below the level of detection (less than 0.4 pg/ml at that time).\textsuperscript{12} This might be due to the lower initial values of plasma Ang II in the experiments performed in normal volunteers without prior furosemide treatment. More likely, our results might demonstrate that, with the administered doses of renin inhibitor, complete suppression of the renin-angiotensin system could not be achieved, despite a fall in PRA below the detection limit of the method.

Another feature that differentiates the changes in PRA and Ang II is the rapid rise in Ang II toward initial values after discontinuation of the renin-inhibitor infusion. Plasma Ang II was no longer different from its initial level 30 minutes after the end of the infusion of 0.125 and 0.250 mg/kg. Although the results given by the two methods of investigation, PRA and plasma Ang II, exhibit roughly a parallel time course, plasma Ang II returned more rapidly to its initial value than PRA. It is logical to assume that measures of Ang II in plasma denote the action of the renin inhibitor more appropriately than determination of PRA. The plasma half-life of this compound is extremely short, and therefore the rapid return of plasma Ang II to normal values is not surprising but contrasts with the slow normalization of PRA. Indeed, the dissociation between plasma Ang II and conventionally measured PRA, using angiotensinase inhibitors instead of Ang I trapping antibodies, was much more pronounced during a similar infusion of CGP 38560 performed in normal volunteers on a normal sodium diet. In those experiments, as well as in the present one, plasma concentration of Ang II recovered rapidly after the infusion was stopped, whereas PRA remained inhibited by 90% 150 minutes after the end of the 0.250 mg/kg infusion. The results obtained with this renin inhibitor, characterized by its strong binding to plasma proteins and its fast metabolism, suggest that the measurement of plasma Ang II is better suited to estimate the blockade of the renin-angiotensin system and to monitor its duration than the in vitro measurement of PRA. Moreover, the use of a trapping assay does not overestimate the duration of action of a renin inhibitor on plasma Ang II as much as the conventional assay of PRA, which was widely used in the past to test renin inhibitors in animals.\textsuperscript{13-15}

Another major result of this study is the observation of dose-dependent increase in active renin during the infusion of the renin inhibitor. This reactive renin release has been observed in monkeys\textsuperscript{16} and in humans,\textsuperscript{2} but the technique available at that time only allowed for the measurement of total renin.\textsuperscript{17} The present use of an immunoradiometric assay that specifically recognizes active renin in plasma\textsuperscript{8} has made it possible to show an immediate and dose-dependent increase of active renin. The measurement of active renin is certainly an index of the feedback efficacy of Ang II on the juxtaglomerular cells and may be an index of titration of renin inhibition although this reactive renin release is quite variable from one individual to another. The small number of subjects studied does not allow a sensitive statistical analysis, but it is interesting to observe that the highest dose of the renin inhibitor still increased active renin more than the 0.125 mg/kg dose. This suggests that the 0.250 mg/kg dose of this renin inhibitor, a maximal dose selected after toxicological studies were performed in two species at 3 mg/kg (CIBA-GEIGY, unpublished data), may not have provided a maximal inhibition of the renin-angiotensin system despite an undetectable PRA, which could be in agreement with the observed submaximal plasma Ang II reduction already discussed.

The plasma half-life of renin is longer than that of Ang II,\textsuperscript{18} and therefore, active renin returned more slowly toward its initial value. In parallel with the metabolism of the renin inhibitor, this reactive increase in active renin induces a resetting of the renin-angiotensin system during renin inhibition. This resetting is similar to that observed with converting enzyme inhibitors. The increase in renin release might limit the efficacy of renin inhibition, and the long-term consequences of a permanent stimulation of renin release by the juxtaglomerular cells are still not known.
The measurement of total renin is less discriminating than the active renin measurement in establishing a dose–response curve. Because inactive renin is not measured directly but is calculated from the results of two different immunoradiometric assays, the accuracy of the results is dependent on the coefficients of variation of both measurements. Therefore, the small decrease in inactive renin is neither statistically nor technically significant. It is likely that an increase in active renin levels occurred in the absence of a change in prorenin, which has been observed with all acute stimuli so far tested, and it represents the release of preformed active enzyme that is stored in secretory granules.

In conclusion, CGP 38560A was found to be a potent inhibitor of human renin even though, at the doses used, maximal inhibition of Ang II generation was not achieved. Its intravenous infusion was well tolerated by normal volunteers. It lowered PRA and Ang II and increased active renin in a dose-dependent manner. Blood pressure, heart rate, and urinary electrolyte excretion remained unchanged. Even if its very short half-life and its minimal bioavailability can be considered major obstacles to its further clinical development, the study allowed a precise analysis of the biochemical parameters that are relevant to monitor this new approach to inhibit the renin-angiotensin system.

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References

Key Words: angiotensin II • angiotensin I • plasma renin activity • renin • renin inhibitors • converting enzyme inhibitors
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