Effects of Renin-Angiotensin System Blockade in Guinea Pigs

Murielle Veniant, Jean-Paul Clozel, Patrick Hess, and Walter Fischli

The goal of the present study was to compare the hemodynamic and biochemical effects of the renin inhibitor Ro 42-5892, the angiotensin converting enzyme inhibitor cilazapril, and the angiotensin II receptor blocker EXP132, the aldehyde derivative of DuP 753. The three drugs were evaluated in guinea pigs, previously treated with furosemide, using their maximal effective doses. Cilazapril decreased arterial blood pressure more than Ro 42-5892 and EXP132. In contrast, Ro 42-5892 and EXP132 had similar effects. The larger decrease of arterial pressure induced by cilazapril was not due to a larger decrease of angiotensin II in plasma and was not influenced by cyclooxygenase inhibition with indomethacin or by bradykinin antagonism with Hoe 140. After binephrectomy, most of the blood pressure-lowering effect of Ro 42-5892 disappeared. In contrast, cilazapril was still markedly effective, pointing to extrarenal effects. We conclude that in furosemide-treated guinea pigs, as opposed to previously published animal models, the decrease of arterial pressure induced by angiotensin converting enzyme inhibitors may be partly due to extrarenal effects not related to the renin-angiotensin system. (Hypertension 1992;19:255-262)

KEY WORDS • renin inhibitors • angiotensin converting enzyme inhibitors • angiotensin II • blood pressure • angiotensin I • guinea pig studies

The renin-angiotensin system (RAS) is a multiregulated proteolytic cascade that produces the potent pressor and aldosterogenic peptide angiotensin II (Ang II). Renin selectively cleaves angiotensinogen, its protein substrate, to release the decapeptide angiotensin I (Ang I), which in turn is processed by angiotensin converting enzyme (ACE) to the octapeptide Ang II.

Although the exact role of RAS in blood pressure homeostasis is not fully elucidated, inhibition of ACE has been proven to be effective in the treatment of hypertension and congestive heart failure.1,2 However, since ACE is a nonselective enzyme that cleaves bradykinin in addition to Ang I,2 certain side effects seen with ACE inhibitors, such as cough or angioneurotic edema, may be intrinsically linked to ACE inhibition.4,5 Thus, inhibition of the extremely specific enzyme renin6,7 or direct blockade of the Ang II receptors8-10 might be a valuable alternative to the inhibition of ACE.

Recently, Ro 42-5892 has been described as a potent and orally active inhibitor of human renin.11 Interestingly, it is also a potent inhibitor of guinea pig renin (IC50, 10 nM) and can thus be used in this animal. DuP 753 has been characterized as a potent and specific Ang II receptor antagonist.12,13 We have used its aldehyde derivative (EXP132) since we have found that in rats, it is more potent than DuP 753 after intravenous administration. Cilazapril is a long-acting ACE inhibitor15,16 also active in guinea pigs.

Methods

General

Studies were performed in adult guinea pigs (Füllinsdorff Albino JPF) weighing 350–400 g. The guinea pigs from each experimental group were maintained under identical conditions and had free access to normal food and water. The guinea pigs received 3 mg/kg furosemide (Lasix, Hoechst-Pharma AG, Frankfurt, FRG) by intramuscular injection 20 minutes before the experiment; they were all then anesthetized with a mixture containing 30% ketamin hydrochloride (Ketavet 10%, Parke-Davis, Berlin, FRG), 20% xylazine (Rompun 2%, Bayer, Leverkusen, FRG), and 50% NaCl (0.9%) also given by intramuscular injection (3 ml/kg body wt).

Bilateral Nephrectomy

Bilateral loin incisions were made under the same anesthesia (as described under “General”). Then both kidney pedicules were ligated, and the two kidneys were withdrawn. When the arterial pressure was measured 1 hour after binephrectomy, the guinea pigs were still anesthetized. When the arterial pressure was measured 24 hours after binephrectomy, the guinea pigs were allowed to recover and were once more anesthetized for...
hemodynamic measurements. Because of their very high sensitivity to anesthesia, guinea pigs binephrectomized for 24 hours had to be tracheotomized and ventilated for the measurement of arterial pressure.

**Hemodynamic Measurements**

The right and the left jugular veins were cannulated with two polyethylene catheters (PE 20) for infusion of drugs. Two catheters were implanted to avoid the mixture of drugs in the same catheters. Mean arterial pressure was measured in the aorta using a 3F high fidelity microtip pressure transducer (Millar SPR 249 A, Houston, Tex.) introduced through the right carotid artery. Heart rate was derived from the blood pressure trace. All the variables were registered on a physiological recorder (Watanabe model WR 3101, Tokyo) after a stabilization period of 20 minutes after surgery. Preliminary experiments have shown that blood pressure and heart rate are stable during a 90-minute period using this experimental procedure. Therefore, all the experiments were performed in less than 90 minutes.

**Ex Vivo Measurements**

To avoid blood pressure changes due to blood withdrawal, measurement of plasma parameters were performed in separate groups of anesthetized guinea pigs (5–6 animals per group and time point). Blood samples (1–2 ml) were obtained from the carotid artery for the parallel measurement of plasma renin concentration (PRC), immunoreactive angiotensin I (irAng I), and immunoreactive angiotensin II (irAng II) and were applied to the carotid artery. After washing with 5 ml of 0.1 M sodium phosphate, pH 7.4. The cartridges were conditioned with 5 ml methanol and equilibrated with 0.1 M sodium phosphate, pH 7.4. The peptides were eluted with 2 ml methanol in polypropylene tubes and divided in two aliquots representing 125 μl plasma extract. The extracts were centrifuged at 3,000g at 4°C for 10 minutes. Angiotensins were extracted immediately from plasma aliquots.

**Extraction of Angiotensins**

Extraction of Ang I and Ang II was achieved by solid-phase extraction using Sep-Pak C18 cartridges (Waters Chromatography Div., Schlieren, Switzerland). The samples were centrifuged at 30°C under reduced pressure in a Vortex evaporator (Buchler Instruments, Fairfield, N.J.). The extraction recoveries of labeled angiotensins from guinea pig plasma (9.5 pg/ml). In contrast, irAng II was estimated with a sensitive polyclonal antibody (Ang I-AS L2) kindly provided by Professor Joel Ménard, INSERM U36, Paris. The IC50 value (50% displacement of the iodinated tracer in the standard curve) was found to be 7.6±0.55 fmol/assay tube (n=10). The cross-reactivities were: Ang I, 100%; human tetra-decapeptide renin substrate (Ang I-val-Ile-His-Thr), 0.84±0.10%; Ang II, less than 0.1%; and Ang III, less than 0.1%. The polyclonal antibody used for the measurement of Ang II (Ang II-AS 923) was raised at Hoffmann-La Roche. The IC50 value was 5.5±0.31 fmol/assay tube (n=8), and the cross-reactivities were: Ang II, 100%; Ang I, 0.37±0.10%; Ang I (2–10), less than 0.02%; Ang III, less than 0.02%; Ang II (3–8), less than 0.02%; Ang II (4–8), less than 0.02%; and Ang II (5–8), less than 0.02%. Thus, these Ang II antibodies are not only remarkably sensitive but also very specific. Neither the Ang I nor the Ang II antiserum cross-reacted with Ro 42-5892, cilazapril, or the Ang II receptor antagonist up to 10⁻⁶ M.

To estimate blanks for the radioimmunoassay (RIA), Ang I and Ang II immunoreactivity was measured in plasma samples collected from binephrectomized guinea pigs that had been treated in addition with a combination of cilazapril and Ro 42-5892 (1 mg/kg/hr and 3 mg/kg/hr, respectively). It was assumed that Ang I and Ang II under these conditions were zero and that, therefore, the remaining immunoreactivity may be considered as a blank subtracted from all the measured values. For both RIA, this blank was relatively high in guinea pig plasma (9.5 pg/ml). In contrast, irAng II measured by the same methodology in plasma samples from squirrel monkeys treated with a combination of cilazapril and Ro 42-5892 was found to be less than 1 pg/ml (unpublished observation from our laboratory).

Finally, a good correlation could be found between irAng I and irAng II in plasma samples of untreated animals (r=0.738; p<0.001), which suggests that the methodology of measuring Ang I and Ang II is valid.

**Chemicals**

Ro 42-5892, (S)-α-[(S)-α-[(tert-butylsulfonyl]-methyl] hydrocinnamamido]-N-[(15,2R,3S)-1-(cyclo-hexylmethyl)-3-cyclopropyl-2,3-dihydroxypropyl]imidazole-4-propionamide methanesulfonate (1:1), DuP 753, and EXP132, the potassium salt of 2-n-butyl-4-chloro-1-(2'-(1H-tetrazol-5-yl)-4-biphenyl)methyl]imidazole-5-carboxaldehyde, were synthesized at F. Hoffmann-La Roche Ltd, Basel. The chemical structure of the Ang II receptor antagonist DuP 753 is 2-n-butyl-4-chloro-5-[4-(2'H-tetrazol-5-yl)-5-phenyl-4-methyl]imidazole-5-carboxaldehyde, potassium salt. The bradykinin antagonist Hoe 140, d-Arg, [Hyp, Thi, d-Tic, d-Oic]-bradykinin (BK) was generously given by Hoechst AG,
FIGURE 1. Line graphs show dose–response curves of increasing doses of Ro 42-5892 (n=6), cilazapril (n=3), EXP132 (n=3), and DuP 753 (n=4) on mean arterial pressure (MAP) and heart rate (bpm). Beats per minute. Dotted lines indicate maximal effective doses chosen for further experiments as described in this study.

We have used the guinea pig since it is responsive to all three types of drugs used. However, the animals had to be anesthetized for blood pressure measurements since they are sensitive to stress. On the one hand, anesthesia is known to modify the reflex control of the hemodynamic status; thus, the effects of the three drugs have been modified. On the other hand, the use of anesthesia with very stable blood pressure allows small changes of arterial pressure to be observed and avoids most of the reflex counterregulations that could interfere with the peripheral vasodilatation induced by the drugs. In addition, the animals were treated with furosemide; this is known to induce a high renin status and to potentiate the effects of ACE or renin inhibitors on blood pressure.

Cilazapril decreased arterial pressure significantly more than Ro 42-5892 and EXP132. EXP132 and Ro 42-5892 both induced the same decrease in arterial pressure. In addition, similar results were obtained when EXP132 was replaced by DuP 753. To explain this difference, we measured the biochemical consequences induced by the three drugs. Both Ro 42-5892 and cilazapril decreased significantly iAng II by 80% and 83%, respectively, but not to zero, even though maximally effective doses were applied. Interestingly, cilazapril did not decrease iAng II more than Ro 42-5892 even though blood pressure was decreased more. The decrease of iAng II induced by Ro 42-5892 was associated with a concomitant decrease of iAng I. In contrast, cilazapril alone increased markedly iAng I, which demonstrates the blockade of ACE. Interestingly, there was a further slight decrease of iAng II when cilazapril was given on top of Ro 42-5892. This could suggest an incomplete blockade of renin even though
the dose was sufficient to maximally reduce blood pressure with the renin inhibitor. Indeed, this is underlined also by the slight increase of irAng I after the addition of cilazapril, which would again point to some remaining renin activity. An alternative explanation would be the existence of a non-renin Ang I–producing enzyme that is not blocked by the renin inhibitor. However, the additional blood pressure decrease by cilazapril could not be explained by the additional blockade of the RAS since similar further reduction of irAng II by the secondary treatment with Ro 42-5892 after cilazapril seemed not to reduce blood pressure any further. Interestingly, similar results of additional biochemical blockade by combined renin and ACE inhibitor treatment without further hemodynamic consequences have been obtained in rats and dogs. This may be explained by a more extensively blocked extraplasmatic than plasmatic RAS, assuming that a substantial formation of plasmatic Ang I from an extraplasmatic compartment exists. Indeed, such an extraplasmatic contribution was shown recently in hypertensive patients. Moreover, we demonstrated that in squirrel monkeys, Ro 42-5892 acts mainly by inhibiting extraplasmatic renin, leading to persistently decreased blood pressure and Ang I as well as Ang II but only to transiently inhibited plasma renin. Thus, the non-equal decrease of plasma renin (PRC) and irAng I seen in this study may be due to tissular effects of Ro 42-5892 in guinea pigs.

**FIGURE 2.** Bar graph shows effects of Ro 42-5892, EXP132, and cilazapril given alone on mean arterial pressure (MAP). In the control, all the baseline values of arterial pressure have been pooled. Cilazapril decreased significantly more arterial pressure than Ro 42-5892 and EXP132. *p<0.05 versus control group, n, Number of guinea pigs. Baseline MAPs were 49±2, 48±3, and 51±1 mm Hg in the Ro 42-5892, cilazapril, and EXP132 groups, respectively.

**FIGURE 3.** Bar graphs show effects of the four drugs given first alone and then in combination. Five to six guinea pigs were used for each group. **p<0.01 versus blood pressure after first treatment. MAP, mean arterial pressure.
TABLE 1. Biochemical Effects of the Three Different Compounds

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>( n )</th>
<th>( \text{irAng I (pg/ml)} )</th>
<th>( \text{irAng II (pg/ml)} )</th>
<th>( \text{PRC (ng Ang I/ml/hr)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
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<td>165±24</td>
<td>191±33</td>
<td>78±10</td>
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<tr>
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<td>145±48</td>
<td>15±9*</td>
<td>120±32</td>
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<tr>
<td><strong>Baseline</strong></td>
<td>5</td>
<td>481±84*</td>
<td>4±2*</td>
<td>90±25</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>5</td>
<td>209±55</td>
<td>52±14</td>
<td>5±1*</td>
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<tr>
<td><strong>Baseline</strong></td>
<td>5</td>
<td>394±52*</td>
<td>3±1*</td>
<td>39±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Results are given as net values where blanks-values were subtracted from the measured immunoreactivity (see "Methods"). irAng I, immunoreactive angiotensin I; irAng II, immunoreactive angiotensin II; PRC, plasma renin concentration; Cila, cilazapril.

EXP132 induced increases of irAng I, irAng II, and PRC. However, these increases of irAng I and PRC were smaller than with cilazapril. In agreement, cilazapril added to EXP132 increased irAng I and PRC further to levels similar to those measured with cilazapril alone. This could mean that receptor blockade does not influence the feedback system regulating the secretion of renin in the same way as the reduction of Ang II or that the blockade of the second Ang II receptor, which is not blocked by DuP 753, would be needed for a maximal renin release.

Since both Ro 42-5892 and EXP132 decreased arterial pressure to the same extent, it is likely that their effect was due to a maximal blockade of the Ang II–mediated vasoconstriction. Therefore, the larger effect of cilazapril was not due to its interaction with RAS. Thus, we evaluated the effects of binephrectomy, since the kidney is known to be the major source of renin. After binephrectomy, the effect of Ro 42-5892 was largely decreased even though there was some residual activity under these conditions. In contrast, the effect of cilazapril was much less decreased, pointing to an extrarenal effect. However, the maximal effective doses have been determined for blood pressure, and we cannot exclude that the maximum effective doses with regard to the biochemical effects are the same.

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Bar graph shows influence of indomethacin on arterial blood pressure decrease induced by Ro 42-5892 alone or in combination with cilazapril (\( n=5 \)). **p<0.01 versus renin inhibitor group. MAP, mean arterial pressure.

![Figure 5](http://hyper.ahajournals.org/)

**Figure 5.** Bar graphs show influence of Hoe 140 on the arterial blood pressure decrease induced by Ro 42-5892 alone or in combination with cilazapril (panel A) (\( n=6 \)). ***p<0.001 versus renin inhibitor group. Panel B: Effect of Hoe 140 on blood pressure decrease induced by cilazapril (\( n=5 \)). MAP, mean arterial pressure.
Frankfurt, FRG. Ro 42-5892, cilazapril, and Hoe 140 were dissolved in normal saline. Propylene glycol (40%) was necessary to dissolve EXP132. However, less than 40 µl of propylene glycol was needed in each animal (such doses of propylene glycol were shown in preliminary experiments not to interfere with the measurements of arterial pressure).

Study Design and Statistical Analysis
In preliminary experiments dose–response curves were constructed for the renin inhibitor, the ACE inhibitor, and the angiotensin II antagonists DuP 753 and EXP132 to determine the maximal effective dose for each compound. In the further experiments, these maximal effective doses were used. Blood pressure reductions induced by the maximal effective doses of the drugs were compared by evaluating the effects of each drug alone or in combination with a second one. This design allowed us to avoid misinterpretation caused by intraindividual variability. Ro 42-5892 and cilazapril were given as slow infusion, whereas EXP132 was given as a bolus injection. Blood pressure reduction was measured and blood samples were withdrawn in separate experiments. Blood sampling and blood pressure measurement were done when blood pressure reduction was maximal (after 10 minutes, 15 minutes, and 20 minutes for Ro 42-5892, cilazapril, and EXP132, respectively). When drugs were combined, the same delay was allowed to measure the maximal effect of the addition of the second drug. To confirm the results obtained with EXP132, a series of experiments were performed where cilazapril was given in addition to DuP 753.

The contribution of the cyclooxygenase pathway in drug effects was assessed by injection of 1 mg/kg i.v. of indomethacin (maximal tolerated dose in guinea pigs) before drug administration.

The role of Bradykinin may be investigated by Hoe 140, a new bradykinin antagonist. 5 µg/kg i.v. has been shown to inhibit completely the bronchopulmonary effect of bradykinin in guinea pigs. In our hands, this dose shifted the dose–response curve of intra-arterially injected bradykinin in guinea pigs by a factor of 10 (data not shown). Two series of experiments were performed with Hoe 140. In six guinea pigs, Hoe 140 was given as an intravenous bolus injection (5 µg/kg) after maximal blood pressure decrease with Ro 42-5892 and 10 minutes before cilazapril infusion. Then, in five guinea pigs the same dose of Hoe 140 was injected after reaching the maximal blood pressure decrease with cilazapril.

Finally, binephrectomized animals were used to clarify the role of the kidney. These animals received either Ro 42-5892 or cilazapril 1 or 24 hours after binephrectomy. All data are expressed as mean±SEM. The effects of the different interventions were compared by an unpaired Student’s t test or by a Dunnett t test (for the binephrectomy experiments). Levels of p<0.05 were considered statistically significant.

Results

Maximal Effective Doses
The four drugs dose-dependently decreased mean arterial pressure (Figure 1). The plateau effect was reached with doses of 3 mg/kg/hr, 1 mg/kg/hr, and 3 mg/kg for Ro 42-5892, cilazapril, and EXP132 or DuP 753, respectively. Therefore, these three doses were chosen for further experiments. The decrease of mean arterial pressure was not associated with a reflex tachycardia. There was a slight decrease in heart rate with all four drugs.

Hemodynamic Effects
The maximal effects on mean arterial pressure of the three drugs given alone were compared (Figure 2). Cilazapril decreased mean arterial pressure more than Ro 42-5892, EXP132, or DuP 753. This difference was confirmed by the experiments in which the drugs were used in combination. Cilazapril increased the effect of Ro 42-5892 (Figure 3A). In contrast, Ro 42-5892 did not lower blood pressure further when given after cilazapril (Figure 3B). Ro 42-5892 did not increase the effect of EXP132 (Figure 3C), and EXP132 did not increase the effect of Ro 42-5892 (Figure 3D). However, cilazapril increased significantly the effects of EXP132 (Figure 3E) and DuP 753 (Figure 3F).

Biochemical Changes
High basal irAng I and irAng II values were found in anesthetized guinea pigs (Table 1) that match the levels reported from anesthetized rats. In the control group, all the variables increased slightly with time during the experiment. However, all changes due to the drugs were compared with the changes observed in the control group. As expected, Ro 42-5892 decreased in parallel PRC, irAng I, and irAng II. The addition of cilazapril decreased irAng II further and increased irAng I slightly. Cilazapril alone decreased markedly irAng II and increased irAng I and PRC. The addition of Ro 42-5892 further decreased the levels of irAng I, PRC, and also irAng II. EXP132 increased all the measured variables. The addition of cilazapril to EXP132 markedly reduced irAng II and interestingly increased further PRC and irAng I levels.

Effects of Cyclooxygenase Inhibition
The inhibition of cyclooxygenase with indomethacin did not prevent the decrease of arterial blood pressure caused by Ro 42-5892 (Figure 4). Moreover, even after indomethacin, cilazapril further decreased arterial pressure after Ro 42-5892.

Effects of Bradykinin Inhibition
The inhibition of bradykinin by Hoe 140 had no effect on the decrease of arterial pressure induced by Ro 42-5892. Cilazapril still further decreased significantly arterial pressure when given after renin inhibitor and bradykinin antagonist (Figure 5A). In addition, Hoe 140 did not increase arterial pressure when given after cilazapril infusion (Figure 5B).

Effects of Binephrectomy
Binephrectomy markedly reduced the effect of Ro 42-5892 (Figure 6, upper panel). In contrast, more than 55% of the effect of cilazapril was still present 24 hours after binephrectomy (Figure 6, lower panel).

Discussion
In the present study, the RAS was blocked at the different levels by specific pharmacological blockers, which should allow conclusions to be drawn concerning the in vivo role of the different components of the RAS.
Inhibition of ACE seems to have an additive effect over renin inhibition or blockade of the Ang II receptors. Clinical studies comparing these three pharmacological interventions are mandatory to establish if these differences are also observed in hypertensive patients.

One hypothesis was that inhibition of ACE stimulated the secretion of cyclooxygenase derivatives. However, indomethacin did not alter the difference between cilazapril and Ro 42-5892 therefore suggesting no activated cyclooxygenase pathway. Another assumption was to explain the additional effect of cilazapril with an interference with the kinin-kallikrein system. Since ACE is known to cleave bradykinin, its inhibition can lead to an accumulation of bradykinin. The absence of effect of the bradykinin antagonist Hoe 140 on the decreased blood pressure induced by cilazapril suggests that bradykinin accumulation may also not be responsible for the additional decrease of arterial pressure over renin inhibitor and Ang II antagonist. However, Hoe 140 is a peptidic antagonist, most likely staying in plasma. Therefore, we cannot exclude that a tissular accumulation of bradykinin may be responsible for the larger effect of cilazapril. Some experimental studies have suggested that bradykinin accumulation could explain part of blood pressure lowering induced by ACE inhibitors. Other studies have shown that ACE inhibitors have a similar effect as renin inhibitors. In addition, we have shown that Ro 42-5892 decreases arterial blood pressure in squirrel monkeys to the same extent as cilazapril. Ang II receptor antagonists have also recently been shown to have similar effects on blood pressure as ACE inhibitors or renin inhibitors in different rat models.

In conclusion, the results of our study suggest that, at least in the present model, inhibiting the RAS through inhibition of renin, ACE, or blockade of the Ang II receptor with the available pharmacological tools does not lead to the same blood pressure decrease. Inhibition of ACE seems to have an additive effect over renin inhibition or blockade of the Ang II receptors. Clinical studies comparing these three pharmacological interventions are mandatory to establish if these differences are also observed in hypertensive patients.

References


FIGURE 6. Bar graphs show influence of binephrectomy on the decrease of mean arterial pressure (MAP) induced by Ro 42-5892 (upper panel) or cilazapril (lower panel). *p<0.05 versus control.


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