Ro 42-5892 Is a Potent Orally Active Renin Inhibitor in Primates

Walter Fischli, Jean-Paul Clozel, Khalid El Amrani, Wolfgang Wostl, Werner Neidhart, Heinz Stadler, and Quirico Branca

The goal of the present study was to characterize the new renin inhibitor Ro 42-5892 in vitro and in vivo. In vitro, Ro 42-5892 inhibited purified human renin and human plasma renin specifically with an IC₅₀ of 0.7 nM and 0.8 nM, respectively. In vivo, Ro 42-5892 reduced mean arterial blood pressure in sodium-depleted marmosets and squirrel monkeys with as low a dose as 0.1 mg/kg orally. Higher doses reduced pressure by 30–35 mm Hg in both species. The duration of blood pressure decrease with 3 mg/kg orally was more than 24 hours. Maximal changes of plasma renin activity, immunoreactive angiotensin I, and immunoreactive angiotensin II were observed at 15 minutes. Renin was reduced by 74±31%, angiotensin I by 85±14%, and immunoreactive active renin was increased by 70±39%. However, unlike pressure, these maximal effects were only transient with complete recovery of renin at 60 minutes under still reduced levels of angiotensin I (61±24%) and angiotensin II (71±38%) and increased concentrations of active renin (86±30%). The blood pressure lowering was due to specific renin inhibition as exemplified by the influence of the kidney, sodium status, species, or stereoselectivity. Moreover, the reduction of arterial blood pressure was similar to the action of the angiotensin converting enzyme inhibitor cilazapril and was not associated with reflex tachycardia in contrast to the pure vasodilator minoxidil. We conclude that Ro 42-5892 is a potent orally active renin inhibitor acting mainly by inhibition of renin in an extraplasmatic compartment. (Hypertension 1991;18:22-31)

The renin-angiotensin system (RAS) is a multiregulated proteolytic cascade that produces the potent pressor and aldosteronogenic peptide angiotensin II (Ang II). Renin selectively cleaves its protein substrate angiotensigen 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 the 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. Moreover, apart from their acute effects, ACE inhibitors may also regress vascular hypertrophy, which parallels hypertension and may thus lead to improved hemodynamics on chronic treatment. Finally, ACE inhibitors might also have antiproliferative activity.

However, since ACE is a rather nonselective enzyme that cleaves bradykinin in addition to Ang I, certain side effects seen with ACE inhibitors such as cough or angioneurotic edema may be intrinsically linked to all ACE inhibitors. Thus, inhibition of the extremely specific enzyme renin might be a valuable alternative to the inhibition of ACE.

Considerable progress has been made in synthesizing potent and orally active renin inhibitors. At present, the two most prominent members seem to be A 64662 and CGP 38560 A. In clinical trials A 64662 was shown to be effective in hypertensive patients after intravenous administration; it was never reported to have been tested orally. CGP 38560 A was shown to lower blood pressure less than the ACE inhibitor captopril in hypertensive patients even after intravenous administration. Thus, an orally active renin inhibitor that is also sufficiently antihypertensive is still lacking.

In the present study, we characterize a new compound that is presently under clinical evaluation. Ro 42-5892 is a potent and selective renin inhibitor in vitro and in vivo. However, the lowering of blood pressure is not strictly paralleled by the changes of the different parameters characterizing the RAS.
Methods

In Vitro Experiments

The in vitro potency of the compound was evaluated in plasma of various species and against purified human renal renin. The selectivity was evaluated against bovine cathepsin D, porcine pepsin, and human plasma ACE.

The plasma assay consisted of 1) 225 µl EDTA-plasma, 2) 10 µl of 8-hydroxyquinoline sulfate in water (0.3 M), 3) 12 µl sodium phosphate (0.1 M), pH 7.4, and 4) 13 µl of various concentrations of Ro 42-5892 in water. Specifically, solutions 2 and 3 were premixed and centrifuged and 25 µl supernatant was added to plasma before the addition of the renin inhibitor dilutions. The resultant pH of the mixture was 6.0 and stable during the subsequent incubation. The samples were then incubated at 37°C for 1–3 hours. The enzymatic activity of renin was estimated by measurement of the produced Ang I, which was quantified by a commercially available radioimmunoassay kit for the determination of Ang I (Clinical Assays, Cambridge, Mass.). As in all the other in vitro assays, the percentage of inhibition was calculated at each concentration point, and the concentration of renin inhibitor that inhibited enzymatic activity by 50% (IC50) was determined.

For use in the in vitro assay, renin was purified in one step from infarcted human kidneys by immunoaffinity chromatography as described.15 The material so prepared was used directly for in vitro assays and had a specific activity of 200 Goldblatt units (GU)/mg protein.

The buffer used for the in vitro assay was 0.1 M sodium phosphate, pH 7.4, containing 0.1% bovine serum albumin and 1 mM disodium-EDTA. The incubation mixture consisted of 1) 100 µl renin in assay buffer (0.5 mGU/ml), 2) 30 µl of human tetradecapeptide renin substrate in 10 mM HCl (30 µM), 3) 10 µl hydroxyquinoline sulfate in water (30 mM), 4) 145 µl assay buffer, and 5) 15 µl renin inhibitor in water at various concentrations. The samples were incubated at 37°C for 3 hours. The generated Ang I was measured as described above in the plasma assay.

Inhibition of bovine spleen cathepsin D and porcine stomach pepsin (both from Sigma Chemical Co., St. Louis, Mo.) was determined in formate buffer at pH 2.8 using (methyl-14C)methemoglobin (NEN chemicals GmbH, Dreieich, FRG) as substrate similar to Hille at al.16 After the addition of Ro 42-5892 in various concentrations, the samples were incubated for 1 hour at 37°C. Then, the nondigested proteinic material was precipitated by trichloroacetic acid (7.5%) and an aliquot of the supernatant counted in a liquid scintillation (β) counter.

Inhibition of human plasma ACE was estimated according to the method of Cushman and Cheung,17 modified by Natoff et al,18 using [14C]chippuryl-histidyl-leucine as the substrate (NEN chemicals GmbH). The activity without addition of Ro 42-5892 was compared with the values in the presence of various concentrations of the renin inhibitor.

Hemodynamic Experiments

The effect of Ro 42-5892 on arterial pressure and heart rate was measured in normotensive marmosets and squirrel monkeys of either sex weighing 300–400 g and 400–700 g, respectively. Sodium-depletion was achieved by injecting 5 mg/kg furosemide subcutaneously 66, 42, and 18 hours before the experiments. Ro 42-5892 (as the methanesulfonate) was given in water by an infant feeding tube (Pharmaseal, Don Baxter S.p.A., Trieste, Italy) to the animals, which were kept in a fasting condition overnight.

Arterial pressure was monitored in the conscious state in unrestrained, chronically instrumented monkeys using a telemetry system developed in our laboratory.19 At least 1 week before the experiment the monkeys were anesthetized, and a 3F high-fidelity pressure tip transducer (Millar Instruments Inc., Houston, Tex.) was inserted into the abdominal aorta through the right femoral artery. Then the catheter was tunneled subcutaneously to the back of the monkey in the interscapular region. The proximal part of the catheter was connected to a transmitter located in a jacket worn by the monkey. The blood pressure signal was transmitted continuously to a receiver, which transformed the signal to an analogue value of blood pressure. Both phasic and mean arterial pressure were recorded. Heart rate was derived from the phasic blood pressure tracing. With this system, it was possible to record arterial pressure for up to 24 months in the same monkey. During the recording period, the monkeys were kept in a separate room without human presence to avoid pressure changes secondary to stress.

For dose titration, oral doses from 0.1 to 10 mg/kg Ro 42-5892 dissolved in 1 ml drinking water were given in random order to separate groups of sodium-depleted monkeys.

Ex Vivo Experiments

Blood sampling. In separate groups of furosemide-treated conscious squirrel monkeys (without telemetry systems, 5–12 animals per group and time point), measurement of plasma parameters was performed after a single oral administration of Ro 42-5892 (10 mg/kg) or placebo. Blood samples (4–5 ml per time point and group) were obtained by direct puncture from the femoral vein for the parallel measurement of plasma renin activity (PRA) and immunoreactive angiotensins (irAng I and irAng II) and were added directly to prechilled tubes containing disodium-EDTA (10 mM final concentration). The blood samples were centrifuged at 3,000g at 4°C for 10 minutes to separate the plasma. A plasma aliquot for the measurement of PRA was immediately frozen and stored at −20°C until use, and two aliquots for the measurement of the angiotensins were extracted immediately. The addition of o-phenanthroline to the angiotensin plasma samples to prevent in vitro
degradation of the peptides was omitted since o-phenanthroline interfered in the radioimmunoasays. However, plasma samples treated with 1.25 mM o-phenanthroline gave the same Ang II values, after deduction of the interference, as the untreated plasma aliquots. This demonstrates that no degradation of Ang II occurs in the absence of o-phenanthroline when the plasma is extracted immediately.

**Extraction of angiotensins.** Extraction of Ang I and Ang II was achieved by solid-phase extraction using Sep-Pak C18 cartridges (Waters Associates, Milford, Mass.). The cartridges were conditioned with 5 ml methanol and equilibrated with 5 ml of 0.1 M sodium phosphate, pH 7.4. The plasma samples (500 μl) were then applied to the cartridges. After washing with 5 ml of 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 250 μl plasma extract. The extracts were evaporated at 30°C under reduced pressure in a vortex evaporator (Buchler Instruments, Fairfield, N.J.). The extraction recovery of labeled angiotensins from the Sep-Pak cartridge under these conditions was: 125I-Ang I, 95.2±0.9% (n = 10) and 125I-Ang II, 92.6±0.6% (n = 10).

**Measurement of plasma renin activity.** For the determination of PRA, the trapping methodology of Poulsen and Jörgensen was used. The method measures renin activity by a radioimmunochemical microassay based on Ang I trapping by antibodies. The adapted method and the generously donated antibodies are described in detail by Nussberger et al.

**Measurement of immunoreactive angiotensins.** Plasma irAng I and irAng II were quantified after immediate extraction of plasma as described above. IrAng I was estimated with a sensitive polyclonal antibody (Ang I-AS L2) produced and generously donated by INSERM U36, Paris. The IC50 value (50% displacement of the iodinated tracer in the standard curve) was found to be at 7.6±0.55 fmol/assay tube (n=10). The cross-reactivities against other angiotensin peptides were: Ang I, 100%; human tetradecapeptide renin substrate (Ang I-Val-He-His-Thr), 0.84±0.10%; Ang II, 0.1% and angiotensin III (Ang III), <0.1%.

The polyclonal antibody used for the measurement of irAng II (Ang II-AS no. 923) was raised at F. Hoffmann-La Roche Ltd, Basel, Switzerland. The IC50 value was 5.5±0.31 fmol/assay tube (n=8) and the cross-reactivities against other angiotensin peptides were: Ang II, 100%; Ang I, 0.37±0.10%; Ang I(2-10), <0.02%; Ang III, <0.02%; Ang II(3-8), <0.02%; Ang II(4-8), <0.02%; Ang II(5-8), <0.02%. Thus, these Ang II antibodies are not only highly sensitive but also specific in an unprecedented manner.

Ro 42-5892 did not cross-react with either antiserum up to 10 μM.

**Measurement of active renin.** Active renin was measured in separate groups of animals, according to the method of Menard et al., with an immunoradiometric assay commercially available at Diagnostics Pasteur, Marnes-La-Coquette, France. The monoclonal antibody used to detect active renin specifically recognizes an epitope of mature renin and does not cross-react with inactive prorenin but does cross-react fully with active renin even when inhibited. Ro 42-5892 interfered slightly in this assay. When the renin inhibitor was added at various concentrations to plasma samples, the value for active renin increased dose-dependently to double the value at 10-6 M of Ro 42-5892. However, the interference was less than 10% at 10 nM, a plasma concentration that is 10 times higher than the IC50 value measured in the plasma assay, leading to a virtually 100% PRA inhibition.

**Experiments Assessing In Vivo Specificity of Ro 42-5892**

Six types of experiments were performed to prove that Ro 42-5892 was affecting blood pressure by a specific blockade of renin. 1) The blood pressure effects of Ro 42-5892 (10 mg/kg oral) were compared in sodium-replete and sodium-depleted monkeys. 2) The blood pressure effects of maximal effective oral doses of Ro 42-5892 (10 mg/kg) and the ACE inhibitor cilazapril (30 mg/kg) were compared in parallel groups of sodium-depleted monkeys. In addition, cilazapril was given in the same monkeys after a maximally effective dose of Ro 42-5892. 3) The blood pressure effects of Ro 42-5892 were assessed in conscious rats and dogs since the renin in these two species is much less potently inhibited in vitro. In rats, arterial pressure was measured directly through a catheter implanted inside the abdominal aorta and tunneled subcutaneously to the interscapular region. In dogs, arterial pressure was measured indirectly on a carotid loop. Intravenous bolus doses of 3 and 10 mg/kg and 0.3, 1, and 3 mg/kg were given to rats (n = 3) and dogs (n = 3), respectively, and arterial pressure was monitored for 30 minutes after each dosing. 4) The blood pressure effect of Ro 42-5892 was assessed in binephrectomized monkeys. Marmosets were binephrectomized 30 hours before and treated with furosemide (5 mg/kg i.m.) 24 hours and 1 hour before administration of Ro 42-5892. Arterial pressure was measured directly in the femoral region under anesthesia (thiobutabarbital, 10 mg/kg i.p.). Control marmosets without binephrectomy (n = 4), also treated in the same way as the binephrectomized animals, were compared with the effect of Ro 42-5892. Two intravenous doses of 0.1 and 1 mg/kg Ro 42-5892 were given consecutively to show that maximally effective doses were used. 5) The effects of two stereoisomers of Ro 42-5892 (Ro 42-5893 and Ro 42-9067), which inhibit human renin less than Ro 42-5892 (IC50 of 34 nM and 20 nM, respectively), were tested in sodium-depleted monkeys and compared with the effect of the same dose of Ro 42-5892. In addition, an oral dose of 10 mg/kg Ro 42-5892 was given in the monkeys that did not respond to the isomers to
In vitro studies characterize Ro 42-5892 as a highly potent and specific renin inhibitor with a molecular weight of 631 and the structure (S)-α-[(S)-α-[tert-butylsulfonyl]-methyl]hydrocinamido]-N-[1S,2R,3S]-1-(cyclohexylmethyl)-3-cyclopropyl-2,3-dihydroxypropyl]imidazole-4-propionamide (Figure 1). The methanesulfonate is soluble in buffers of pH 5.5 or lower (>200 mg/ml) but less soluble at physiological pH (<2 mg/ml).

In vitro studies characterize Ro 42-5892 as a highly potent and specific renin inhibitor (Table 1). It inhibited purified human renin at pH 7.4 with an IC₅₀ of 0.7×10⁻⁴ M. Similarly, the compound inhibited human plasma renin at pH 6.0 with an IC₅₀ of 0.8×10⁻⁵ M and was roughly equipotent in marmoset (IC₅₀, 1.0×10⁻⁹ M) and squirrel monkey (IC₅₀, 1.7×10⁻⁹ M) plasma. In contrast, the inhibitor was 150- and 5,000-fold less potent in dog (IC₅₀, 1.1×10⁻⁷ M) and rat (IC₅₀, 3.6×10⁻⁸ M) plasma, respectively. Furthermore, Ro 42-5892 displayed a considerable specificity and discriminated the closely related enzymes porcine pepsin and bovine cathepsin D from human renin by a factor of more than 50,000 (IC₅₀, 2.4×10⁻⁴ M and 3.5×10⁻⁵ M, respectively). Nonrelated enzymes such as human plasma ACE were not inhibited at concentrations up to 100 μM.

In vivo, Ro 42-5892 was given orally to sodium-depleted marmosets and squirrel monkeys (Figure 2) to establish the dose-response relation. In both monkey species, even the low oral dose of 0.1 mg/kg decreased mean arterial pressure (MAP) by 20–25 mm Hg. Blood pressure reduction was enhanced with increasing oral doses of the renin inhibitor. In marmosets, the maximal decrease of MAP was already reached with 1 mg/kg, which decreased MAP by 30 mm Hg. In squirrel monkeys, blood pressure decreases of 35 mm Hg were found with 3–10 mg/kg. Heart rate was not affected by the treatment.

In a separate experiment, the duration of the blood pressure response was established in sodium-depleted marmosets using the oral dose of 3 mg/kg Ro 42-5892 (Figure 3). In the control group, the daily activity cycle of the animals was reflected by changes of MAP. It was higher during daytime and lower during nighttime. In the treated group, MAP fell by 30 mm Hg and followed the MAP pattern of the untreated group on this lower level. After 24 hours, MAP was still reduced to the same extent as after the onset of the effect. A similar effect was reached with the ACE inhibitor cilazapril at an oral dose of 30 mg/kg.

The effects of Ro 42-5892 on the plasma RAS parameters were studied in sodium-depleted squirrel monkeys with the single oral dose of 10 mg/kg Ro 42-5892. The biochemical RAS parameters were measured in plasma of vehicle- and drug-treated animals at time 0, 15, 60, and 240 minutes after oral administration (Table 2). In the control groups, there was a fluctuation of the mean values between the groups at the different time points due to a relatively high interanimal variation. Nevertheless, the change at a certain time point was strictly estimated by comparison with the respective control group at this time point. At 15 minutes, plasma renin activity, irAng I, and irAng II were reduced by 73±31%, 85±14%, and 89±17%, respectively, but were still measurable. At 60 minutes, PRA was not inhibited anymore, although irAng I and irAng II were still measurable.

![Chemical structure of Ro 42-5892.](http://hyper.ahajournals.org/)

**TABLE 1. Inhibitory Potency and Selectivity of Ro 42-5892**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Species</th>
<th>Assay (pH)</th>
<th>IC₅₀ (nM)</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure renin</td>
<td>Human</td>
<td>7.4</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma renin</td>
<td>Human</td>
<td>6.0</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Marmoset</td>
<td>6.0</td>
<td>1.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Squirrel monkey</td>
<td>6.0</td>
<td>1.7</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>6.0</td>
<td>107</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>6.0</td>
<td>3,600</td>
<td>5,140</td>
<td></td>
</tr>
<tr>
<td>Pepsin</td>
<td>Porcine</td>
<td>2.8</td>
<td>240,000</td>
<td>343,000</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>Bovine</td>
<td>2.8</td>
<td>35,000</td>
<td>50,000</td>
</tr>
<tr>
<td>Plasma ACE</td>
<td>Human</td>
<td>7.4</td>
<td>&gt;100,000</td>
<td>&gt;140,000</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme.
reduced by 61±24% and 71±38%, respectively. At 240 minutes, similar to the 60-minute time point, irAng I and irAng II were still reduced by 58±20% and 68±21%, respectively, without a decrease of PRA. In separate groups of monkeys, the effect on active renin was estimated and found to be increased at all time points compared with the control group, with a maximal effect at 60 minutes. This increase, which was statistically not significant, was still present at 240 minutes and was paralleled by the decreased irAng I and irAng II levels.

The assessment of the in vivo specificity of Ro 42-5892 by six different types of experiments points to a blood pressure decrease that is mediated by specific inhibition of the RAS. Sodium depletion with furosemide markedly enhanced the effects of Ro 42-5892 on blood pressure. With 10 mg/kg oral dosing, the drop of arterial pressure was 15 mm Hg and 30 mm Hg in marmosets without and with sodium depletion, respectively (Figure 4). In sodium-depleted monkeys, Ro 42-5892 and cilazapril decreased arterial pressure to the same extent with maximal effective oral doses as reported above (Figure 3). Furthermore, cilazapril did not decrease arterial pressure further after administration of Ro 42-5892 (Figure 5). In contrast to its effect in monkeys, Ro 42-5892 did not decrease arterial pressure in rats and dogs with intravenous doses up to 10 mg/kg (data not shown) or in binephrectomized monkeys (Figure 6). The two less active stereoisomers of Ro 42-5892 (Ro 42-5893 and Ro 42-9067) were virtually devoid of blood pressure-lowering activity at the intravenous dose of 1 mg/kg in contrast to Ro 42-5892. In addition, Ro 42-5892 given to the same monkeys after the isomers reduced arterial pressure markedly (Figure 7). Finally, Ro 42-5892
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![Graph showing effects of Ro 42-5892 and cilazapril on mean arterial pressure](image)

**Figure 3.** Line graphs show effects of Ro 42-5892 (3 mg/kg p.o., n=4) and cilazapril (30 mg/kg p.o., n=4) on mean arterial pressure (MAP) in conscious sodium-depleted marmosets. The two drugs were compared with a saline control (n=3). Beginning of experiment was 8:00 AM. MAP was lower at nighttime (time 12-24 hours) in the three groups compared with daytime.

did not induce a reflex tachycardia in contrast to the pure vasodilator minoxidil, despite a similar drop of arterial pressure (Figure 8).

**Discussion**

Ro 42-5892 appears to be a potent and specific inhibitor of human renin. It is specific for the primate enzyme and recognizes much less renin from dog and rat, thereby limiting the in vivo experiments to pri-

mates. A similar specificity has been obtained with numerous other renin inhibitors and is due to the species difference in the structure of renin. Other members of the aspartyl protease family, to which renin belongs, such as pepsin or cathepsin D are inhibited only with very high concentrations of Ro 42-5892. Human plasma ACE was not inhibited even at 100 μM. Such specificity as described here for Ro 42-5892 is thought to be mandatory for any clinical application to avoid unwanted side effects.

The measurement of arterial pressure using a telemetry system allows continuous recording of pressure in conscious, unrestrained monkeys. Ab-

![Graph showing effects of Ro 42-5892 on mean arterial pressure in conscious marmosets with and without sodium depletion by furosemide treatment](image)

**Figure 4.** Line graph shows effects of Ro 42-5892 on mean arterial pressure in conscious marmosets with (n=10) and without (n=6) sodium depletion by furosemide treatment. **p<0.01 between the two groups.

### Table 2. Effect of Oral Ro 42-5892 (10 mg/kg) in Sodium-Depleted Squirrel Monkeys

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>MAP (mm Hg)</th>
<th>PRA (pg Ang I/ml/hr)</th>
<th>AR (pg/ml)</th>
<th>irAng I (pg/ml)</th>
<th>irAng II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>90±6 (n=5)</td>
<td>1,190±280 (n=12)</td>
<td>59±8 (n=10)</td>
<td>46±6 (n=11)</td>
<td>56±12 (n=12)</td>
</tr>
<tr>
<td>15 minutes</td>
<td>V 93±7 (n=5)</td>
<td>1,360±390 (n=10)</td>
<td>43±7 (n=10)</td>
<td>48±7 (n=10)</td>
<td>46±8 (n=10)</td>
</tr>
<tr>
<td></td>
<td>D 79±2 (n=9)</td>
<td>360±160* (n=6)</td>
<td>73±15† (n=6)</td>
<td>7±2* (n=6)</td>
<td>5±2† (n=6)</td>
</tr>
<tr>
<td>60 minutes</td>
<td>V 91±6 (n=5)</td>
<td>1,840±310 (n=5)</td>
<td>72±10 (n=6)</td>
<td>61±12 (n=6)</td>
<td>65±20 (n=5)</td>
</tr>
<tr>
<td></td>
<td>D 60±3 (n=9)</td>
<td>1,760±370 (n=6)</td>
<td>134±17§ (n=6)</td>
<td>24±4† (n=6)</td>
<td>19±3† (n=6)</td>
</tr>
<tr>
<td>240 minutes</td>
<td>V 91±5 (n=5)</td>
<td>1,500±400 (n=6)</td>
<td>38±12 (n=5)</td>
<td>58±10 (n=5)</td>
<td>56±12 (n=6)</td>
</tr>
<tr>
<td></td>
<td>D 58±3 (n=9)</td>
<td>1,190±180 (n=12)</td>
<td>99±35 (n=12)</td>
<td>24±7§ (n=12)</td>
<td>18±2‡ (n=12)</td>
</tr>
</tbody>
</table>

Results are mean±SEM. Numbers in brackets refer to number of animals. Data were analyzed with Student's t test for unpaired samples. MAP, mean arterial pressure; PRA, plasma renin activity; AR, active renin; irAng I, immunoreactive angiotensin I; irAng II, immunoreactive angiotensin II; V, vehicle; D, drug.

* p<0.077; § p<0.05; † p<0.01; ‡ p<0.001.
sence of anesthesia is important since it has been shown to modify cardiac reflexes. However, small monkeys are difficult to train and are very sensitive to stress. Any human presence may interfere with the recording of arterial pressure. Thus, the use of telemetry for measuring arterial pressure in these small monkeys was needed. Under these conditions, oral administration of Ro 42-5892 potently reduced arterial blood pressure in marmosets and squirrel monkeys. Both monkey species behaved similarly.

The measurement of the biochemical parameters could not be performed in the same monkeys that were used for the recording of arterial blood pressure. Because the animals were not catheterized, any blood withdrawal would have interfered with the blood pressure recordings. Furthermore, in these small monkeys, only small volumes of blood could be withdrawn. Thus, we had to choose between measuring all the parameters at one time point or one parameter in the same animals at all the time points. Although the latter possibility was more intriguing for a time-course experiment as described here, we have chosen the former approach, which did allow evaluation of the relation between the variables.

For measuring PRA we used the trapping methodology of Poulsen and Jørgensen since this technique avoids the addition of angiotensinase inhibitors such as hydroxyquinoline, phenylmethylsulfonyl fluoride, or diisopropyl fluorophosphate to the plasma but protects Ang I from degradation by the addition of Ang I antibodies during the in vitro production of Ang I. The in vitro condition used to generate Ang I comes thus as close as possible to a physiological condition. Furthermore, it is a well-described standard technique that is used presently for the evaluation of Ro 42-5892 in humans in the laboratories of Brunner and Nussberger (Camenzind et al). Using this technique allows a direct comparison with clinical results. Discrepant results have been obtained in human volunteers treated with the renin inhibitor CGP 38560A when PRA inhibition was evaluated with different methodologies. Only the trapping methodology, which is used here, was shown to give inhibitions of PRA that were parallel to the reductions in Ang I.

Rather high basal values of irAng I and irAng II are measured in the squirrel monkeys. Although the angiotensins were measured without prior high-performance liquid chromatography separation, good correlations could be shown between PRA and irAng I as well as between irAng I and irAng III using the samples from untreated squirrel monkeys, thus suggesting no major technical problems in measuring PRA, irAng I, and irAng II (see following paragraph, Figure 9). The lack of major nonspecific cross-react-
tivities in plasma is demonstrated by the low levels of angiotensins measured during renin inhibition by Ro 42-5892 (Table 2).

After oral treatment of squirrel monkeys with 10 mg/kg Ro 42-5892, PRA was decreased; however, the decrease was not statistically significant. This decrease was associated with a decrease of irAng I and irAng II in plasma and with an increase of active renin. Similar results have been obtained in normal volunteers and hypertensive patients after oral administration with 600 mg of Ro 42-5892.30,31 The decrease of irAng I and irAng III is the direct consequence of renin inhibition, whereas the increase of active renin reflects the interruption of a negative feedback mechanism. Ang II is known to inhibit the release of renin from the juxtaglomerular cells where it is produced.32 Interestingly, the functional effects of renin inhibition, such as the effects on irAng I, irAng II, active renin, and blood pressure, were much longer lasting than PRA inhibition. A more detailed demonstration of this nonparallel effect appears in the correlation plots (Figure 9). PRA correlated with irAng I in plasma samples of untreated ($r=0.55; p<0.002$) and treated ($r=0.65; p<0.001$) animals but with a different regression ($y=0.0166x+27.6$ versus $y=0.0175x+2.7$) having similar slopes but significantly different elevations ($p<0.001$). IrAng I and irAng II correlated also ($r=0.77; p<0.001$), but no change in correlation is seen between untreated and treated animals. This would suggest that renin is still inhibited after 60 minutes but in an extraplasmatic compartment, which is of prime importance for the functional effects. Indeed, extraplasmatic functional renin was demonstrated recently in hypertensive humans where a high percentage of the plasma Ang I was shown to be produced locally and not by plasma renin.33 Thus, renin inhibition in plasma seems not to be crucial for the function of Ro 42-5892 in the squirrel monkey.

Moreover, there was also no strict correlation between blood pressure and irAng I and irAng II.
(compare Figure 2B and Table 2). At 15 minutes, the angiotensins were reduced maximally with a nonmaximal blood pressure decrease. In contrast, at 60 minutes and 240 minutes, blood pressure was reduced maximally with nonmaximally decreased angiotensins. This suggests that local rather than plasmatic levels of angiotensins are major determinants for blood pressure regulation and, again, that Ro 42-5892 exerts its physiological effects mainly in an extraplasmatic compartment.

Nonparallelism between blood pressure decreases and reductions in biochemical parameters has been observed before (for review, see Reference 10), but in contrast to the present case, blood pressure reduction after acute treatment with other renin inhibitors was only transient with more effective reductions of biochemical parameters (mostly PRA). We may speculate that Ro 42-5892 behaves differently with regard to pharmacokinetics from other renin inhibitors. Indeed, recent evidences suggest that the reference renin inhibitors CGP 38560 A and A 64662 have a volume of distribution that is equal to the blood volume. In contrast, the volume of distribution for Ro 42-5892 was shown to be at least 10 times the blood volume (unpublished observation from our laboratory). Thus, Ro 42-5892 is concentrated mainly in tissue, whereas the other two inhibitors stay in plasma. How this different pharmacokinetic behavior translates to differences in mode of action is not yet clear.

There is evidence that the RAS may operate both as a systemic endocrine and a tissue autocrine/paracrine system. All the components of the RAS are found in kidney, blood vessel, heart, adrenal, and brain (for review, see Reference 36). There is speculation that the local RAS may influence functions such as vascular tone and structure, cardiac contractility and mass, as well as renal hemodynamics. We have to suggest that the local action of Ro 42-5892 may be mainly in kidney since blood pressure effects are abolished by binephrectomy.

An alternative explanation for these nonparallel effects induced by Ro 42-5892 may be nonspecific effects of the compound. However, experiments designed to answer this question showed that Ro 42-5892 most likely acted specifically by blocking renin. The potentiation of blood pressure reduction with Ro 42-5892 by furosemide is known to occur also with ACE inhibitors and is explained by an activation of the RAS by low sodium. In addition, Ro 42-5892 did not also lower blood pressure on top of the ACE inhibitor cilazapril, which points to a common mechanism of action, which is the reduction of Ang II. Moreover, Ro 42-5892 did not reduce arterial pressure in rats and dogs, which suggests that the drug needs to be able to inhibit renin to act. This was not possible at these doses because the compound inhibits rat and dog renin much less than primate renin. The absence of effects in binephrectomized marmosets suggested that Ro 42-5892 is acting on a factor, which is probably renin, that is in or derived from kidney. Moreover, in these experiments we used maximally effective doses of Ro 42-5892, as shown by the absence of additional effects of 1 mg/kg i.v. over 0.1 mg/kg i.v. The ineffectiveness of closely related substances on blood pressure, such as the two stereoisomers Ro 42-5893 and Ro 42-9067 with less affinity to renin, shows that the renin inhibitory activity of the compounds is essential for its in vivo activity. Finally, the absence of reflex tachycardia, similar to what has been described for ACE inhibitors, showed that Ro 42-5892 did not act as a pure vasodilator, which would have induced tachycardia. The absence of reflex tachycardia with ACE inhibitors has been explained by a suppression of the facilitatory effect of Ang II on the baroreceptor reflex. Thus, the results of these six types of experiments strongly suggest that Ro 42-5892 acts specifically by inhibition of renin.

In conclusion, Ro 42-5892 is a potent, specific, and orally active renin inhibitor that is able to reduce blood pressure in marmosets and squirrel monkeys. Extraplasmatic renin inhibition might explain its long duration of blood pressure decrease. Preliminary clinical results obtained in hypertensive patients seem to confirm these results.

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References


**Key Words** • renin inhibitor • blood pressure • angiotensin I • angiotensin II • renin • plasma renin activity
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