Monoclonal Antibodies Against Human Renin
Blood Pressure Effects in the Marmoset
JEANETTE M. WOOD, CHRISTOPH HEUSSER, NEELAM GULATI,
PETER FORGIARINI, AND KARL G. HOFBAUER

SUMMARY The in vivo effects of two anti–human renin monoclonal antibodies with a high binding affinity for primate renin were studied in conscious, volume-depleted marmosets. These antibodies, R-3-17-7 and R-3-36-16, both have high binding activity for renin, but only R-3-36-16 inhibits the enzymatic activity of renin in vitro. In vivo, R-3-17-7 did not affect blood pressure after intravenous injection of doses up to 100 μg/kg, although plasma renin activity was partially reduced. In contrast, R-3-36-16 induced a reduction in blood pressure and an inhibition of plasma renin activity at a threshold dose of 3 μg/kg. The maximum fall in blood pressure and complete inhibition of plasma renin activity were observed after R-3-36-16, 10 μg/kg; these effects persisted for up to 2 hours. Pretreatment with a converting enzyme inhibitor or nephrectomy prevented the hypotensive effects of R-3-36-16. Conversely, pretreatment with R-3-36-16 prevented the hypotensive effects of a converting enzyme inhibitor. These findings indicate that the hypotensive response induced by R-3-36-16 is due entirely to blockade of the renin-angiotensin system. Thus, R-3-36-16 appears to be a specific, potent, and long-acting inhibitor of primate renin. Such monoclonal antibodies provide interesting tools for studying the effects of acute and chronic renin blockade. (Hypertension 8: 600–605, 1986)

KEY WORDS • renin inhibition • converting enzyme inhibition • blood pressure • primates • teprotide • enalaprilat

The renin-angiotensin system plays an important role in blood pressure (BP) regulation and sodium homeostasis.1–3 This has stimulated interest in agents that inhibit renin activity. Recently, most efforts in this area have concentrated on synthetic inhibitors of renin.4,5 An alternative approach is the use of antibodies as inhibitors.6–7 One of the first attempts to block the biological effects of renin with antibodies was made by Johnson and Wakerlin in 1940.8 In this and other studies,5–13 passive and active immunization against renin indicated the antihypertensive potential of renin inhibition. However, the results of these early studies had to be interpreted with caution because of the impurity of the renin preparations used for immunization and the unknown specificity of the antisera.

More recently, antisera have been raised against pure preparations of canine,14 mouse,15 and human renin.6,16 A disadvantage of antisera for in vivo studies is that serum contains substances other than the antibodies that might influence the biological response. This problem was partly overcome by purification of the immunoglobulin (Ig) G fractions from antisera against canine renin.17 However, the supply of a particular antisera is limited, and antibodies from different antisera have highly variable binding affinity and selectivity. Monoclonal antibodies produced by the hybridoma technique18 have the advantage of infinite supply and precise characteristics. Anti-canine renin monoclonal antibodies have been produced, but none of them inhibited the enzymatic activity of renin.19 However, it has been possible to obtain monoclonal antibodies against human renal renin that do inhibit its enzymatic activity.6,20-22

We have prepared several monoclonal antibodies against pure human renin that, to our knowledge, express the highest binding affinity for renin reported to date.21 In the present study, the in vivo effects of two of these antibodies were compared. Both antibodies have a high binding activity for human renin, but only one inhibits the enzymatic activity of renin in vitro. Since

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Received October 2, 1985; accepted January 8, 1986.

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these antibodies do not react with renin from nonprimat species, a primate was used for the in vivo studies. A quantitative analysis was made of the effects of the antibodies on blood pressure and plasma renin activity (PRA) in conscious, normotensive marmosets after acute volume and sodium depletion.

Materials and Methods

Monoclonal Antibodies

The monoclonal antibodies against renin were selected and characterized, as will be described in detail in a separate publication. Briefly, BALB/c mice were immunized with a purified preparation of human kidney renin. The mouse spleen cells were then fused with the nonsecretory myeloma cell line SP2/0. Antibody-producing cells were identified by the demonstration of renin binding and renin inhibitory activity in the supernatants. Cells were cloned and recloned by limiting dilution; the selected hybridomas were grown intraperitoneally in pristane-treated BALB/c mice, and the immunoglobulin was isolated according to a modification of the procedure of Parkam et al. After removal of cells by centrifugation, ascites was ultracentrifuged for 1 hour at 70,000 g to remove lipids and cell debris. The euglobulin fraction was obtained by precipitation with 50% saturated ammonium sulfate at 4°C, followed by dialysis against 20 mM tris(hydroxymethyl)aminomethane (Tris)/50 mM NaCl (pH 7.9). Immunoglobulins were then purified on diethylaminoethyl cellulose (DE52) by elution with 20 mM Tris/80 mM NaCl (pH 7.9), and the peak containing the monoclonal antibody was concentrated by ultrafiltration on a PM 30 membrane. The final preparation was at least 95% pure as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

The binding activity of the monoclonal antibodies was determined in a solid-phase assay using renin adsorbed on microtiter plates (20 ng of renin per well) as well as in a solution assay using 125I–labeled human renin (2.5 ng in 50 μl phosphate-buffered saline containing 1% bovine serum albumin). IC50 = concentration required to achieve 50% of maximal inhibition of renin activity in human (renin-free plasma with the addition of purified human kidney renin, 250 pg/ml or marmoset (1:50 dilution) plasma. Plasma renin activity was similar in human and marmoset incubation mixtures (35 and 32 ng angiotensin I/ml/hr). Dash indicates that no inhibition was observed up to the highest concentration tested (10−6 M).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BC50 (M) Human renin</th>
<th>IC50 (M) Human renin</th>
<th>Marmoset renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOPC-21</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>R-3-17-7</td>
<td>9.5 x 10−11</td>
<td>1.3 x 10−11</td>
<td>1.2 x 10−11</td>
</tr>
<tr>
<td>R-3-36-16</td>
<td>6.2 x 10−11†</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

†BC50 = concentration required to achieve 50% of maximal binding to renin-coated microtiter plates (20 ng of renin per well) or purified 125I–labeled human renin (2.5 ng in 50 μl phosphate-buffered saline containing 1% bovine serum albumin). 

Animals

Male and female marmosets (Callithrix jacchus, Ciba-Geigy, Sisseln, Switzerland) weighing between 300 and 400 g were used. The animals were maintained on a diet that consisted of normal salt pellets (Nafag, Gossau, Switzerland), meat, eggs, fruit, and milk.

Mean arterial BP and heart rate (HR) were measured in conscious, restrained marmosets as described previously. Catheters were implanted into a femoral artery and a tail vein 1 to 2 days before an experiment. The arterial catheter was used for the measurement of BP and HR and the collection of blood samples, and the venous catheter was used for the injection of substances. The PRA was determined as described previously.

Dose-Response Effects of Monoclonal Antibodies

The dose-response effects of the monoclonal antibodies were evaluated in normotensive marmosets after stimulation of renin release by furosemide. The diuretic (5 mg/kg i.v.) was injected 45 minutes before the test substances. Vehicle (0.9% saline) was injected in a volume of 0.5 ml/kg (n = 6); R-3-36-16 was injected in a dose of 0.1 (n = 4), 1 (n = 4), 3 (n = 6), 10 (n = 4), or 100 (n = 5), μg/kg; R-3-17-7 in doses of 100 (n = 6) or 1000 (n = 5) μg/kg; and MOPC-21 in a dose of 100 μg/kg (n = 6). To test the degree of blockade of the renin-angiotensin system, the converting enzyme inhibitor teprotide (2 mg/kg i.v.) was injected 120 minutes after a dose of monoclonal antibody. The BP was recorded continuously and HR was recorded intermittently. Blood samples were collected at various intervals for the determination of PRA.
Specificity of R-3-36-16 in Vivo

To determine whether the hypotensive effects of R-3-36-16 were due entirely to blockade of the renin-angiotensin system, this antibody was administered after a converting enzyme inhibitor. Four furosemide-treated marmosets received the long-acting converting enzyme inhibitor enalaprilat (MK 422; 2 mg/kg i.v.) 120 minutes before R-3-36-16 was injected in a dose of 100 µg/kg. Blood samples were taken for measurement of PRA 0, 30, 60, and 120 minutes after injection of enalaprilat and 30 minutes after injection of R-3-36-16.

To determine whether renin of renal origin was essential for the hypotensive response to R-3-36-16, the BP effects of this antibody were investigated in nephrectomized marmosets. R-3-36-16 was injected in doses of 10, 30, and 100 µg/kg in four marmosets 1 day after bilateral nephrectomy. Blood samples for measurement of PRA were taken before and 1 day after nephrectomy.

Statistics

Values given in the text and figures were means ± SEM. The statistical significance of changes was calculated by paired Student's t test (two sided).

Results

In control experiments, there was a small gradual decrease in BP during the first hour after injection of vehicle alone (Figure 1). The BP remained constant after injection of MOPC-21. Neither of the doses of R-3-17-7 had a significant effect on BP when compared with the effects of vehicle. The lowest dose of R-3-36-16 (0.1 µg/kg) had no effect on BP when compared with that of vehicle (Figure 2). A small but significant decrease in BP was observed 10 minutes after the 1 µg/kg dose. The maximum hypotensive response was obtained with 1000 µg/kg; 100 µg/kg had no further effect (see Figure 2). The BP had recovered to values observed in vehicle-treated marmosets 120 minutes after 3 µg/kg dose, whereas the full response persisted 120 minutes after the 10 and 100 µg/kg doses.

Administration of the converting enzyme inhibitor teprotide after the 1000 µg/kg dose of R-3-36-16 lowered the BP (see Figure 1). Teprotide also lowered BP when administered 120 minutes after the 0.1, 1, and 3 µg/kg doses of R-3-36-16 (see Figure 2). It had no significant effect on BP when injected after 10 and 100 µg/kg doses of R-3-36-16. No significant changes in HR were observed after any of the doses of antibody or teprotide (see Figure 3).

The PRA remained constant in marmosets that received vehicle, whereas it tended to increase in marmosets that received MOPC-21 (see Figure 1). The PRA was reduced to 60% of initial values 30 minutes after the 1000 µg/kg dose of R-3-36-16 and remained decreased at 120 minutes. The 1000 µg/kg dose of R-3-36-16 reduced PRA to 35% of initial values at 30 minutes, although PRA recovered to 60% of initial values at 120 minutes. The 0.1 and 1 µg/kg doses of R-3-36-16 had no significant effect on PRA (see Figure 2); however, PRA was reduced to 25% of initial values 30 minutes after the 3 µg/kg dose of R-3-36-16 and had recovered to 58% of initial values at 120 minutes. The PRA was reduced to less than 10% of initial values 30 minutes after the 10 and 100 µg/kg doses of R-3-36-16 (see Figures 2 and 3); and this inhibition persisted for up to 120 minutes after injection.

The converting enzyme inhibitor enalaprilat lowered BP in furosemide-treated marmosets to a similar extent as the 10 and 100 µg/kg doses of R-3-36-16 (Figure 4). R-3-36-16, injected in a dose of 100 µg/kg 120 minutes after enalaprilat, had no additional effect on BP. The HR was unchanged by administration of enalaprilat and R-3-36-16. The PRA increased approximately twofold after injection of enalaprilat and was unmeasurable after injection of R-3-36-16.

The PRA was 28 ± 7 ng angiotensin I/ml/hr before nephrectomy and was unmeasurable after nephrectomy. R-3-36-16, injected in doses of 10, 30, and 100 µg/kg, lowered BP to a similar extent as the vehicle.
**FIGURE 2.** Mean changes in blood pressure (△BP) and plasma renin activity (△PRA) after i.v. bolus injection of R-3-36-16 in doses of 0.1 (n = 4), 1 (n = 4), 3 (n = 6), 10 (n = 4), or 100 (n = 5) μg/kg. The converting enzyme inhibitor teprotide (2 mg/kg) was injected at 120 minutes. Single (p < 0.005) and double (p < 0.01) asterisks indicate significant difference compared with values at zero minutes. Single (p < 0.05) and double (p < 0.01) stars indicate significant difference compared with values at 120 minutes.

**FIGURE 3.** Mean arterial blood pressure, heart rate, and plasma renin activity in furosemide-treated marmosets after i.v. bolus injection of R-3-36-16 (10 μg/kg; n = 4). The converting enzyme inhibitor (CEI) teprotide (2 mg/kg) was injected at 120 minutes. AII = angiotensin II.

μg/kg, had no effect on BP in nephrectomized marmosets (change in BP at 30 minutes: +3 ± 6, −5 ± 2, and −4 ± 3 mm Hg).

**Discussion**

Our results demonstrate that the monoclonal antibody R-3-36-16 is a potent inhibitor of primate renin, not only in vitro, but also in vivo. In previous in vivo studies with antisera directed against renin, large amounts of undefined proteins were administered. Even in a study with purified IgG, a large amount (8 mg/kg) of a mixed population of antibodies was administered. In the present study, a dose as low as 3 μg/kg of a pure antibody with well-defined characteristics was sufficient to inhibit PRA and lower BP. The maximum effects on BP and PRA were observed after 10 μg/kg, and no additional effects were seen after higher doses. In previous experiments we have shown that this is the maximum fall in BP observed in furosemide-treated marmosets after complete inhibition of the renin-angiotensin system by synthetic inhibitors of renin or converting enzyme.

The fall in BP induced by R-3-36-16 appears to be the specific consequence of renin inhibition. The hypotensive response was not observed after circulating renin had been eliminated by bilateral nephrectomy. In addition, the maximum fall in BP, induced after administration of an effective dose of a converting enzyme inhibitor or R-3-36-16, was similar. Finally, prior administration of a fully effective dose of a converting enzyme inhibitor completely prevented the hypotensive effects of R-3-36-16.

The initial fall in BP reached a plateau within 10 to 20 minutes after injection of R-3-36-16. The further small progressive fall in BP observed after some of the doses of R-3-36-16 probably reflects the spontaneous
Figure 4. Mean arterial blood pressure, heart rate, and plasma renin activity in furosemide-treated marmosets after i.v. bolus injection of the converting enzyme inhibitor (CEI) enalaprilat (2 mg/kg; n = 4). R-3-36-16 (100 µg/kg) was injected at 120 minutes. AI = angiotensin I.

fall in BP observed in vehicle-treated marmosets. The duration of the maximum effect of R-3-36-16 on BP and PRA was dose-dependent. Within 2 hours after injection of the 3 µg/kg dose, BP and PRA had recovered by over 50%, whereas the full effects persisted 2 hours after the 10 and 100 µg/kg doses. The longer duration of action of the higher doses may reflect saturation of the mechanisms responsible for the elimination of the antibody from the circulation. Alternatively, the shorter duration of the lower dose might be due to a compensatory increase in renin secretion. We have previously demonstrated that total immunoreactive renin in the plasma increases two- to threefold after acute inhibition of renin by a synthetic renin inhibitor. Such an increase in renin concentrations may be sufficient to overcome inhibition induced by lower doses of the monoclonal antibody. Unfortunately, the enzyme-linked immunosorbent assay that was used to measure the total amount of renin in the presence of the synthetic renin inhibitor could not be applied in the present study because R-3-36-16 interferes with the assay.

In control experiments, the nonspecific IgG-1M0PC-21, given in doses similar to those of R-3-36-16 and R-3-17-7, did not reduce PRA or BP. Indeed, PRA tended to increase and BP was slightly elevated in comparison to the experiments with vehicle. Although the monoclonal antibody R-3-17-7 has a high binding activity for primate renin, it does not inhibit the enzymatic activity of renin in vitro. In vivo, however, R-3-17-7 appeared to induce a dose-dependent reduction in PRA after intravenous administration. This finding may indicate that renin is removed from the circulation after it has bound to the antibody R-3-17-7. Interestingly, no significant fall in BP was observed, even though PRA was reduced by up to 65% after R-3-17-7. This finding is in contrast to the effects of R-3-36-16 and suggests that the reduction in PRA after R-3-17-7 does not reflect a true inhibition of renin in situ. Measurement of the circulating renin concentrations after administration of R-3-17-7 and R-3-36-16 may help to clarify this point; however, a suitable assay system to measure renin in the presence of these antibodies is not presently available.

The monoclonal antibody R-3-36-16 is the most potent inhibitor of human renin (antibody or synthetic inhibitor) that has been reported so far. Antibodies have the capacity for a large number of intermolecular contacts with their ligand and therefore can have a high affinity for the ligand. This ability probably explains the extremely high potency of R-3-36-16 compared with that of synthetic renin inhibitors. The synthetic renin inhibitor CGP 29 287, evaluated previously under the same experimental conditions as those of R-3-36-16, probably inhibits renin activity by a mechanism different from that of R-3-36-16. CGP 29 287 is thought to act as a mimic of the transition state of angiotensinogen. It probably inhibits renin activity by binding to the active site of renin and thereby preventing the cleavage of angiotensinogen. In contrast, R-3-36-16 may not bind to the active site, since it does not distinguish between active and inactive renin. R-3-36-16 probably binds to an epitope near the active cleft and, by virtue of its large molecular size, prevents the access of angiotensinogen into the active cleft.

Despite the 1000-fold difference in molecular size and a different mechanism of inhibition, R-3-36-16 and CGP 29 287 have similar effects in vivo. However, R-3-36-16 has a greater potency and longer duration of action than CGP 29 287 or any other of the presently available synthetic inhibitors. Thus, R-3-36-16 is an interesting alternative to synthetic renin inhibitors as an experimental tool for physiological studies. However, its potential to induce an immune response, to form complexes, and to activate complement after repeated administration remains to be investigated. Such effects of antirenin monoclonal antibodies would limit their application in long-term studies.
In this and previous studies, we have evaluated the effects of converting enzyme or renin inhibition in the same experimental model. Therefore, we can now make a general comparison of the effects of these different modes of interference with the renin-angiotensin system. In all of our studies, the maximum fall in BP induced after converting enzyme or renin inhibition by various inhibitors was similar and there was no concomitant increase in HR. Since pretreatment with a renin inhibitor completely prevented the hypotensive response to a converting enzyme inhibitor and vice versa, the hypotensive response appears to be due entirely to blockade of the renin-angiotensin system with both types of agent. Kinins do not seem to contribute to the hypotensive response induced by converting enzyme inhibitors in marmosets after mild volume and sodium depletion. The lack of an effect on HR, despite a fall in BP, is consistent with other experimental studies and clinical findings after converting enzyme inhibition. Several studies indicate that angiotensin II potentiates the activity of the sympathetic nervous system and inhibits vagal activity. Disappearance of either of these effects of angiotensin II following inhibition of renin or converting enzyme, might explain the lack of an increase in HR.

In summary, the results of the present study indicate that the monoclonal antibody R-3-17 reduces PRA in vivo, although it does not inhibit primate renin in vitro. This partial reduction in PRA does not result in a hypertensive response in normotensive marmosets after acute volume and sodium depletion. In contrast, the monoclonal antibody R-3-16 is a potent inhibitor of primate renin both in vitro and in vivo. As a consequence of the inhibition of renin, R-3-16 lowers BP without a concomitant increase in HR. This hypertensive response appears to be due entirely to blockade of the renin-angiotensin system. Thus, R-3-16 is a specific, potent, and long-acting inhibitor of primate renin in vivo and provides a suitable tool for studying the therapeutic potential of the immune approach to renin inhibition.

Acknowledgments

We are grateful to Miss Guri Christiansen and Mrs. Michèle Martignot for their technical assistance and to Mrs. Elisabeth Scheidegger for her assistance and care of the marmosets. We thank Miss Susi Naegeli for typing the manuscript.

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Hypertension. 1986;8:600-605
doi: 10.1161/01.HYP.8.7.600

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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