Continuous Versus Intermittent Angiotensin Converting Enzyme Inhibition in Renal Hypertensive Rats

Thierry Battle, Christian Schnell, Bettina Bunkenburg, Didier Heudes, Jeannette M. Wood, Joël Ménard

Converting enzyme inhibitors impair renal function of the kidney beyond a stenosis of the renal artery in humans and induce histological lesions in the clipped kidney of renal hypertensive rats. In two-kidney, one clip hypertensive rats, we compared the time course and magnitude of the biochemical effects of angiotensin converting enzyme inhibition on the plasma renin-angiotensin system, cardiac hypertrophy, renal lesions, and 24-hour blood pressure decrease induced by either intermittent angiotensin converting enzyme inhibition administration (benazepril PO, 10 mg/kg once a day, n=93) or continuous administration (benazeprilat, 3 mg/kg per day via osmotic pumps, n=92). Control rats (n=91) received the drug vehicle intermittently or continuously. Mortality was significantly reduced by both intermittent (n=3/93) and continuous (n=3/92) inhibition compared with controls (n=18/91) (P<.001). Changes in the plasma renin-angiotensin system and blood pressure were parallel. A continuous suppression of the activity of the plasma renin-angiotensin system was associated with a 24-hour decrease in blood pressure with continuous inhibition, whereas intermittent inhibition induced a similar fall in blood pressure only for the first hours after gavage. Heart weight (5.12±0.12, 3.98±0.09, 4.32±0.12 g/kg in controls [n=8], continuous inhibition [n=18], and intermittent inhibition [n=18], respectively) was significantly reduced to the same extent by both treatments (P <.0001), and clipped kidney weight (3.28±0.11, 1.83±0.12, 2.20±0.15 g/kg in controls [n=8], continuous inhibition [n=18], and intermittent inhibition [n=18], respectively) was significantly reduced in both groups of treated rats (P <.0001). After removal of the unclipped kidney, plasma creatinine was significantly increased in both treatment groups (P <.0001) compared with nephrectomized hypertensive control rats but to a significantly greater extent in the continuously inhibited rats (317±17, 317±8, 260±8 µmol/L in controls [n=8], continuous inhibition [n=18], and intermittent inhibition [n=18]; P <.0001). Therefore, changes in blood pressure and the plasma renin-angiotensin system were parallel after either continuous or intermittent inhibition. It was possible to decrease blood pressure continuously during 24 hours only with continuous inhibition. Both treatments reduced heart weight to a similar extent. Damage to the clipped kidney, as revealed by elevated plasma creatinine levels after nephrectomy of the unclipped kidney, was slightly reduced but not avoided by the intermittent inhibition. (Hypertension 1993;22:188-196)

KEY WORDS • hypertension, renovascular • angiotensin converting enzyme inhibition • angiotensin II • rat studies

The intrarenal renin-angiotensin system (RAS) plays a major role in sustaining glomerular filtration rate in situations in which the system has been stimulated, such as sodium depletion, renal artery stenosis, or congestive heart failure.1-2 Under these circumstances, angiotensin I converting enzyme (ACE) inhibition impairs renal function, but the magnitude of the phenomenon, the relative role of the fall in blood pressure (BP), and the blockade of the intrarenal RAS are matters of debate.3 The histological lesions of the clipped kidney in two-kidney, one clip (2K-1C) experimental hypertension4-9 and the functional impairment of a stenotic kidney in humans10,11 have been worsened by the administration of ACE inhibitors. It is not known whether the renal function abnormalities observed in humans and the renal lesions observed in animals are due to the greater fall in BP induced in these situations by ACE inhibitors rather than by other antihypertensive drugs, or whether they are specifically related to the intrarenal blockade of the RAS and the modification of glomerular filtration rate. In another situation, congestive heart failure, in which perfusion pressure and the intrarenal RAS also play a critical role in the maintenance of glomerular filtration rate, the duration of ACE inhibition has been one of the factors suspected to influence the occurrence of a renal deterioration. Packer et al12 found that the longer acting ACE inhibitor enalapril (at a dose of 20 mg orally twice a day) compromised renal function more than captopril.
These doses of benazepril and benazeprilat were chosen as a continuous therapy (continuous inhibition, or continuous ACE inhibition on anatomic and functional status of the clipped kidney).

Methods

Animals

The procedures followed in the care and euthanasia of the study animals were in accordance with the European Community Standards. Six-week-old male Wistar rats (Iffa-Credo, L’Arbresle, France) weighing 150±10 g were used throughout these experiments. The animals received a standard rat chow (NAFAG, Gossau, Switzerland) and tap water ad libitum.

Renal Hypertension

Renal hypertension was induced by the Goldblatt 2K-1C method adapted to the rat. A silver clip (diameter, 0.2 mm) was placed on the left renal artery while the rats were maintained under ether anesthesia. From the third to the fourth week after clipping, systolic blood pressure was measured in conscious animals with a tail cuff method (Aplex BP recorder 8006, Ugo Basile, Comerio, Italy), and body weight was measured twice a week. Of the 328 rats operated, 52 were eliminated from the study because they either failed to develop hypertension within 4 weeks (systolic blood pressure <180 mm Hg, n=43) or they developed malignant hypertension marked by rapid loss of body weight (n=9).

Drug Treatment

Treatment with the converting enzyme inhibitor (CEI) benazepril was started 4 weeks after renal artery clipping with one of the two following modes of application applied to the different groups using a random assignment table (Geigy Scientific Tables, Documenta Geigy, Basel, Switzerland). As an intermittent therapy (intermittent inhibition, II), benazepril hydrochloride (prodrug of the CEI benazeprilat, CIBA-GEIGY Corp, Summit, NJ) was given orally once a day by gavage at a dose of 10 mg/kg in vehicle (distilled water). As a continuous therapy (continuous inhibition, CI), the active CEI benazeprilat was given continuously (3 mg/kg per day) via osmotic minipumps (osmopumps, Alzet 2002, Alza Corp, Palo Alto, Calif) implanted in the peritoneal cavity. As a control (C), another group of hypertensive rats received either the vehicle alone (1 mL/kg per day PO) or via the osmopumps (12 µL/h). These doses of benazepril and benazeprilat were chosen because they gave the same maximum fall of BP in a preliminary dose-finding experiment. Because the guaranteed pumping period for the osmopumps was only 2 weeks, they were replaced after 2 weeks of treatment. On this occasion, the dose was adapted to the increase in body weight. Similarly, the amount of CEI given orally was kept constant for 2 weeks and then adjusted for the increase in body weight after 2 weeks. No further adjustment was made during the following 2 weeks.

Two days before the beginning of treatment, rats from each treatment group were randomly assigned to two subgroups, either BP recording and kidney and heart weight (n=49) or blood collection for day 1 and day 30 at 2, 6, and 24 hours after last drug intake (n=227).

Blood Pressure Measurement

An arterial catheter was implanted in the right femoral artery while the rats were maintained under light anesthesia induced by halothane. The catheter was exteriorized at the back of the neck, protected, and sealed. For the measurement of BP, catheters were connected to a pressure transducer (Statham p32 ID, Gould Instruments, Cleveland, Ohio) while the rat was allowed to move freely in its cage. Lines to the catheter were attached to a swivel and protected by a metal spring. During recording, rats were maintained in individual cages and had free access to food and water. Mean arterial BP and heart rate (HR) were recorded on-line (Data analyzer 32, Buxco Electronics, Sharon, Conn, with an IBM AT03 computer). Blood pressure was recorded over a 24-hour period on the first and last weeks of treatment on the same rats.

After the last BP recording, the right kidney (unclipped kidney) was removed and weighed. Twenty-four hours later, plasma samples were collected with rats under halothane anesthesia for the measurement of urea and creatinine. Thereafter, rats were killed and the clipped kidney and heart were weighed.

Blood Sampling

Serial blood sampling was not possible in the same animal because of the large volume of blood required for all biochemical measurements. Therefore, different groups of rats were used for blood collection at each time point. To avoid variations in the way of collecting samples, we adopted rigorous standardization (same investigator, same depth of halothane anesthesia) for each animal. Samples were collected at 2, 6, and 24 hours after oral drug administration on the first and last (day 30) days of drug administration in each CEI-treated group and the control group. Rats were anesthetized with halothane for blood sampling, and the maximum possible amount of blood was taken by direct puncture of the abdominal aorta. Serum was collected for ACE determinations, and EDA or heparin plasma was collected for the other biochemical parameters. For plasma angiotensin measurements, blood was collected in a cooled syringe containing an inhibitor solution (0.05 mM phenanthroline, 0.003 mM neomycin sulfate, 0.12 M EDTA, 10 µM enalaprilat, and 5 µM CGP 44099A, a renin inhibitor). Samples were immediately centrifuged, and the plasma samples were frozen in ice-cooled.
Nephrectomy of the Clipped Kidneys

To evaluate the excretory functions of the clipped kidney alone without the complication of the unclipped kidney, we removed the latter from the rats. Twenty-four hours later, plasma samples were collected for creatinine and urea determinations with a commercial kit (Roche, Cobas-Bio, Wako Chemical, Japan). During this period, drug administration was maintained.

Biochemical Measurements

Plasma concentrations of benazeprilat were measured by the method of Graf et al.13 After inactivation of endogenous ACE by heating, plasma was incubated with hippuryl-histidyl-leucine as substrate and blank plasma as the source of ACE. Released hippuric acid was measured by high-performance liquid chromatography and determined with a kit (Boehringer Mannheim, France).

Serum converting enzyme activity was measured by incubation of the serum with the substrate hippuryl-glycyl-glycine. The liberated glycyl-glycine was derivatized with a borate-buffered trinitrobenzenesulfonate solution (pH 9.6) and measured spectrophotometrically (420 nm) according to the method of Neels et al.16

Determinations of plasma renin concentration (PRC) were performed by incubation of plasma with an excess of substrate (renin-free substrate from binephrectomized animals) at pH 7.4, 37°C; plasma renin substrate (PRS) concentrations were determined by incubation of plasma with an excess of renin (isolated from mouse submaxillary glands) at pH 7.4, 37°C. In both assays, the plasma concentrations of renin were measured by a radioimmunoassay.17

Plasma concentrations of angiotensin II (Ang II) were measured by the method of Nussberger et al.18 The Ang II was extracted from 500 μL of plasma (Bond Elut, Analytichem, Harbor City, Calif) and then separated from other angiotensins by high-performance liquid chromatography. The fractions containing Ang II were then measured by a radioimmunoassay with a sensitivity of 1 fmol Ang II per fraction. The antibody used was a prediluted IgG-Ang II-1 (IgG Corp, Nashville, Tenn). The cross-reactivity was 63% for Ang II/angiotensin III and 0.1% for Ang II/Ang I.

Histology

After the last day of treatment, kidneys were removed, weighed, and plunged in a Dubosq Brazil’s solution (80% ethanol [66%], picrin [0.5%], formaldehyde [27.5%], acetic acid [6%]). They were kept at 4°C until embedded in paraffin. The histometric study of kidney slices (0.3 μm) was performed on 15 clipped and 15 unclipped randomly chosen kidneys among the treatment groups (C, II, CI). This gave a total of 90 kidneys investigated. The technique of Serra19 was used with a video image analyzer (Nachet, France) quantifying collagen content (red sirius coloration). The ratio of the area of fibrotic tissue to undamaged tissue was measured in 10 areas for each kidney slice, and the average value was taken.

Statistical Methods

Results are expressed as mean±SEM. Parametric values were evaluated according to a two-way analysis of variance with the Scheffe’s coefficient. The Wilcoxon nonparametric test was used to compare biochemical values among the various groups. Paired parameters were analyzed by Friedman’s Test. The χ² test was used to compare mortality among groups. Significance was taken at a value of P<0.05.

Results

Mortality

Among the 276 rats included in the investigation, 24 died before the end of the experiment. The mortality of the renal hypertensive rats was significantly reduced in ACE inhibitor–treated rats compared with the C group (treated, 6/185 [3.24%] versus C, 18/91 [19.78%; P<.001]. There was no significant difference in mortality between the two modes of CEI administration (II, 3/93 versus CI, 3/92). Given the lack of influence of the mode of vehicle administration on BP of C rats, values from these animals have been pooled.

Body Weight

The mean body weight of the different groups was not significantly different at the beginning of the treatment (191±7 [n=91], 197±5 [n=93], and 199±5 g [n=92] for C, II, and CI, respectively). The various treatments had no significant effect on body weight. For the rats kept in the experiment until the fourth week, body weight was 278±4 (n=32), 289±4 (n=35), and 277±2 g (n=33) for C, II, and CI, respectively.

Blood Pressure and Heart Rate

Before drug administration and 4 weeks after left renal artery clipping, the mean arterial pressures of the different groups of rats were 185±4 (n=18), 189±5 (n=19), and 190±5 mm Hg (n=19) for C, II, and CI, respectively. There were no significant differences in the mean values of BP among the different groups. On the first day of treatment, the maximum fall in BP was similar for the two modes of CEI administration (change in mean arterial pressure: −74±7 and −73±7 mm Hg for CI and II, respectively), but the time course of the response was different (Fig 1). With CI (n=19), BP decreased slowly. A maximum effect was obtained after approximately 10 hours, which then persisted for up to 24 hours. With II (n=19), BP decreased more rapidly. A maximum effect was obtained after approximately 4 to 6 hours, which persisted for 6 hours. BP began to recover thereafter but was still significantly lowered compared with pretreatment values and values in the C group after 24 hours (change in mean arterial pressure at 24 hours: −35±7 mm Hg, P<.05). Pretreatment values for HR were similar in all groups (346±8, 332±12, and 344±11 beats per minute for C, II, and CI, respectively), and the various treatments had no significant effect on baseline or diurnal HR rhythm (data not shown).

During a 24-hour cycle on the last day of treatment (day 30), BP had slightly but not significantly increased in the C group (200±9 mm Hg, n=8). BP was not different from the first treatment day in the CI group

acetone and then stored at −20°C. The hearts and kidneys of these animals were removed and weighed.
FIG 1. Line graphs show continuous intra-arterial blood pressure monitoring during 24 hours. Conscious renal two-kidney, one clip hypertensive rats received either 0.9% saline (1 mL/kg per day, control, n=12) (○), benazepril by gavage per os (10 mg/kg per day, intermittent infusion, n=12) (○), or benazeprilat by osmopumps intraperitoneally (3 mg/kg per day, continuous infusion, n=11) (△). MBP, mean blood pressure.

(n=18) and was still significantly decreased compared with pretreatment values and values for the C group on day 30 (ΔBP, −62±7 mm Hg, P<.001). In the II group (n=19), before the daily dose of the CEI, BP was significantly lower than in the C group (ΔBP, −17±7 mm Hg, P<.05) and was much higher than in the CI group. It decreased rapidly (within 1 to 2 hours) after gavage to levels similar to those observed in the CI group and remained at this level for approximately 8 hours (ΔBP compared with C, −52±6 mm Hg). Thereafter, BP gradually returned to the level immediately before last drug intake (ΔBP, −16±3 mm Hg 24 hours after last dose). On the last day of treatment, HR values were significantly (P<.05) lower in both ACE inhibitor-treated groups than in the C group, with no significant difference between CI and II. However, the CEI treatments had no effect on the diurnal HR rhythm.

Benazeprilat Plasma Levels

On the first day of treatment, benazeprilat levels gradually increased over 24 hours in the CI rats (Fig 2). In the II rats, benazeprilat levels were very high after 2 hours and decreased thereafter but were still detectable at 24 hours. Benazeprilat levels were similar in both groups 6 hours after treatment, the time when the fall in BP was maximal. A similar pattern was observed on day 30 in all groups, although benazeprilat concentrations were lower than on the first treatment day.

Heart Weight

At the end of the 1-month treatment period, heart weight (of the rats whose BP had been recorded until the fourth week) was significantly reduced in both treatment groups (5.12±0.12 [n=8], 4.32±0.12 [n=18], and 3.98±0.09 [n=18] g/kg for C, II, and CI, respectively; P<.0001 for either CI or II compared with C) (Fig 3). The heart weight of the II group was not significantly different from that of the CI group. In all the three groups, there was a strong correlation between the heart weight and the area under the curve calculated from the BP recording during 24 hours on day 30; values for the coefficient of correlation (r) were C, 0.97 (n=8); II, 0.74 (n=18); and CI, 0.78 (n=18). In the rats used for the blood collections (no BP recording), the heart weight was 4.95±0.1 (n=32), 4.10±0.04 (n=35), and 3.85±0.08 (n=33) mg/kg for C, II, and CI, respectively; P<.0001 for either CI or II compared with C.

FIG 2. Bar graphs show comparison of plasma benazeprilat levels in renal two-kidney, one clip hypertensive rats that received benazepril by gavage per os (prodrug, 10 mg/kg per day, intermittent infusion) (filled bars) or benazeprilat by osmopumps intraperitoneally (3 mg/kg per day, continuous infusion) (open bars) at 2, 6, and 24 hours after administration. *P<.05.

FIG 3. Plots show effect of angiotensin converting enzyme (ACE) inhibition on cardiac hypertrophy at the end of 1 month of treatment. Ratio of heart weight (HW) to body weight (BW) of renal two-kidney, one clip hypertensive rats after 1 month of treatment is shown. Rats received either 0.9% saline (1 mL/kg per day, control, n=8) (○), benazepril by gavage per os (10 mg/kg per day, intermittent, n=18) (○), or benazeprilat by osmopumps intraperitoneally (3 mg/kg per day, continuous, n=18) (△). Cardiac hypertrophy was significantly reduced in both treatment groups (control, 5.12±0.12; intermittent, 4.32±0.12; continuous, 3.98±0.09 g/kg; P<.0001 for either continuous or intermittent compared with control).
Kidney Weight and Fibrotic Lesions

At the end of the 1-month period of treatment with the CEI, the weight of the clipped kidney was significantly reduced (P<.0001) in both the CI and II groups compared with the C group (3.28±0.11 [n=8], 1.83±0.12 [n=18], and 2.20±0.15 [n=18] g/kg in C, CI, and II, respectively) (Fig 4). The kidney weight of the CI group was not statistically different from the kidney weight of the II group. The weight of the unclipped kidney tended to increase in both treated groups, although the difference was not statistically significant.

Similar results were obtained in the rats used for the blood collections (clipped kidney weight: 3.19±0.09 [n=32], 1.94±0.09 [n=33], and 2.18±0.19 [n=33] g/kg in C, CI, and II, respectively). The difference between the CI and II groups was not statistically significant.

The results of the measurements of renal fibrosis are presented in Table 1. In both groups of ACE inhibitor-treated rats, the percentage of fibrotic lesions tended to increase in the clipped kidney and decrease in the unclipped kidney compared with C. There was a good correlation between kidney weight and the degree of fibrosis in the clipped kidney (r=.59, P<.001, n=45), where lesions were due to ischemia, and not in the unclipped kidney (r=.07, NS, n=45), where lesions were secondary to hypertension.

Plasma Creatinine and Urea Levels

Twenty-four hours after nephrectomy of the unclipped kidney (in the three groups), plasma creatinine concentration was significantly increased in both ACE inhibitor–treated groups compared with the vehicle-treated rats (260±8 [n=18], 317±8 [n=18], and 117±39 [n=8] mmol/L in II, CI, and C, respectively; P<.0001). This increase was significantly greater (P<.0001) in CI than II rats (Fig 4, bottom panel). Plasma urea concentrations tended to increase in both ACE inhibitor–treated groups (41±5 [n=18], 38±2 [n=18], and 24±4 [n=8] mmol/L in II, CI, and C, respectively), but differences were not statistically significant.

Serum and Plasma Renin-Angiotensin System

Results and statistical significance of serum and plasma concentrations of the RAS are shown in Table 2.

Angiotensin converting enzyme activity. On the first day of treatment, serum ACE activity was almost completely inhibited at all time points in the CI group. In the II group, ACE activity was almost completely inhibited 2 and 6 hours after the first oral dose but had recovered partially at 24 hours (68% of inhibition compared with C). A similar pattern was observed on day 30 in both treatment groups, and the value of ACE inhibition at 24 hours for the II group fell to 45%. Note that ACE activity had increased in the C group compared with the vehicle-treated rats (200±8 [n=18], 317±8 [n=18], and 117±39 [n=8] μmol/L in II, CI, and C, respectively; P<.0001). This increase was significantly greater (P<.0001) in CI than II rats (Fig 4, bottom panel). Plasma urea concentrations tended to increase in both ACE inhibitor–treated groups (41±5 [n=18], 38±2 [n=18], and 24±4 [n=8] mmol/L in II, CI, and C, respectively), but differences were not statistically significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Clipped kidney</th>
<th>Unclipped kidney</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CK/BW (g/kg)</td>
<td>Fibrosis (%)</td>
</tr>
<tr>
<td>C</td>
<td>3.08±0.19</td>
<td>3.20±1.44</td>
</tr>
<tr>
<td>II</td>
<td>2.28±0.21*</td>
<td>4.11±1.40</td>
</tr>
<tr>
<td>CI</td>
<td>2.19±0.16*</td>
<td>5.26±1.43</td>
</tr>
</tbody>
</table>

*P<.05 compared with controls.

Fig 4. Plots show effect of angiotensin converting enzyme (ACE) inhibition on clipped kidney (CK) weight and renal function. Ratio of CK weight to body weight (BW) and plasma creatinine concentration was significantly (P<.0001) increased in both ACE inhibitor–treated groups compared with controls. Twenty-four hours after nephrectomy of the undipped kidney (in the three groups), plasma creatinine levels of renal two-kidney, one clip hypertensive rats after 1 month of treatment are shown. Rats received either 0.9% saline (1 mL/kg per day, control, n=8) (○), benazepril by gavage per os (10 mg/kg per day, intermittent, n=18) (○), or benazepril by osmopumps intraperitoneally (3 mg/kg per day, continuous, n=18) (●). CK weight was significantly (P<.0001) reduced in both the continuous and intermittent groups compared with controls. This increase was significantly greater in continuous than in intermittent rats (P<.001).
Table 2. Serum and Plasma Concentrations of Renin-Angiotensin System Parameters Measured on Days 1 and 30

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Intermittent</th>
<th>Continuous</th>
<th>Intermittent</th>
<th>Continuous</th>
<th>Intermittent</th>
<th>Continuous</th>
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<tbody>
<tr>
<td><strong>ACE (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Day 1</td>
<td>225±47</td>
<td>0†</td>
<td>2±1†</td>
<td>6±3†</td>
<td>0†</td>
<td>72±25†</td>
<td>0†‡</td>
</tr>
<tr>
<td>Day 30</td>
<td>608±66</td>
<td>0†</td>
<td>9±3†</td>
<td>10±3†</td>
<td>12±2†</td>
<td>333±58§</td>
<td>9±3†‡</td>
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<tr>
<td><strong>PRC (ng Ang I/mL/h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>420±185</td>
<td>1605±301†</td>
<td>1690±61†</td>
<td>1018±169§</td>
<td>826±69§</td>
<td>230±67</td>
<td>641±106§</td>
</tr>
<tr>
<td>Day 30</td>
<td>76±15</td>
<td>599±103†</td>
<td>479±61†</td>
<td>310±65§</td>
<td>448±75§</td>
<td>59±23</td>
<td>806±124‡</td>
</tr>
<tr>
<td><strong>PRS (ng Ang I/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>1.4±0.2</td>
<td>0.81±0.21</td>
<td>0.78±0.16</td>
<td>0.55±0.1§</td>
<td>1.15±0.1‡</td>
<td>1.04±0.17</td>
<td>1.39±0.22</td>
</tr>
<tr>
<td>Day 30</td>
<td>1.2±0.1</td>
<td>0.93±0.1</td>
<td>0.75±0.12§</td>
<td>0.87±0.09</td>
<td>1.0±0.19</td>
<td>1.28±0.08</td>
<td>0.65±0.11‡</td>
</tr>
<tr>
<td><strong>Plasma Ang II (fmol/mL)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>279±52</td>
<td>49±26†</td>
<td>49±12†</td>
<td>24±6†</td>
<td>16±4†</td>
<td>20±3†</td>
<td>8±6†</td>
</tr>
<tr>
<td>Day 30</td>
<td>60±22</td>
<td>7±3†</td>
<td>3±1†</td>
<td>9±2†</td>
<td>9±6†</td>
<td>20±4§</td>
<td>5±2†‡</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; PRC, plasma renin concentration; Ang I, angiotensin I; PRS, plasma renin substrate; Ang II, angiotensin II. Two-kidney, one clip hypertensive rats received either 0.9% saline (1 mL/kg per day, control), benazepril by gavage per os (10 mg/kg per day, intermittent), or benazeprilat by minipumps intraperitoneally (3 mg/kg per day, continuous). Values are mean±SEM of 10 to 12 values per time point. Because there was no time effect on renin-angiotensin system parameters in control rats, at 2 (n=9), 6 (n=12), and 24 (n=11) hours, all values were pooled between per os and minipump vehicle-treated rats to show difference in the renin-angiotensin system between days 0 and 30.

*P<.001 compared with day 30.
†P<.001 compared with controls.
‡P<.001 compared with intermittent inhibition at corresponding time point.
§P<.05 compared with controls.

Discussion

The intrarenal role of the RAS in maintaining glomerular filtration rate in the presence of a decreased perfusion pressure explains why the interruption of the RAS is more likely than other antihypertensive treatments to impair renal function in situations in which the intrarenal RAS has been activated, such as a stenosis of the renal artery or congestive heart failure. This impairment may also be due to the fact that the blockers of the RAS are more effective than other drugs in controlling BP in these patients and therefore induce a greater decrease of the perfusion pressure of the stenotic kidney. We wanted to induce two different situations of RAS inhibition and analyze their influence on 24-hour BP and on the clipped kidney of the 2K-1C model of renal hypertension. In untreated hypertensive rats, the well-known changes in the plasma RAS were observed, with a shift from a high renin-high Ang II status at the fourth week after clipping toward a normal renin-normal Ang II status at the eighth week after clipping. A rise in plasma ACE was also observed, which may reflect the increased activity of the vascular converting enzyme reported during the chronic phase of this experimental hypertension.

The goal to induce an intermittent or continuous inhibition was achieved, as shown by the totally different time course of the plasma levels of benazeprilat in both groups of treated rats. Two hours after gavage, plasma levels of the active drug were fourfold higher than in animals treated by osmopumps, and they were threefold lower 24 hours after gavage; the rats treated by osmopumps were more constantly exposed to the ACE inhibitor effects than the animals treated by gavage. The biochemical consequences of these two inhibition patterns were investigated by measuring the circulating levels of the RAS parameters. A major fall in plasma Ang II was observed in the two treated groups, which was reduced by 89% at the 24th hour after 30 days of...
continuous inhibition and by only 59% after intermittent inhibition. This major fall in plasma Ang II is observed despite the high levels of plasma Ang II measured in the C rats and is secondary to the method used for blood sampling (anesthesia and aortic puncture). The lowest values were observed for the 24 hours of the 30th treatment day in the group treated by osmopumps. Because plasma Ang II is not the best index of local production of Ang II and most Ang I and Ang II are produced outside of the plasma, the rise in PRC, which reflects the interruption of the intrarenal feedback loop between Ang II and renin release, may represent a more sensitive index of the degree of RAS blockade. Indeed, PRC was much higher in CI rats, at both day 1 and day 30, especially 24 hours after gavage, which demonstrates a more complete and continuous blockade of the RAS in rats treated by osmopumps. The fall in PRC measured by an enzymatic assay is an index of the consumption of angiotensinogen when renin is produced in excess. It was lower at 24 hours on day 30 in rats treated by osmopumps. Therefore, the biochemical plasma parameters indicate a more permanent blockade of the RAS over 24 hours in rats receiving benazeprilat continuously by osmopumps.

To our knowledge, this is the first time that BP has been monitored for 24 hours in this rat model (including plasma Ang II measurements with the appropriate inhibitors to avoid the in vitro generation of angiotensin) and the relation between BP and plasma components of the RAS investigated. A sustained reduction in BP was induced by continuous inhibition. From 9 AM to 6 PM, the maximum fall in BP was similar in both treated groups. BP began to recover thereafter in II rats in parallel with the biochemical changes observed in plasma. BP was still lower than in untreated hypertensive rats, even at 24 hours. Even though the peak effect was similar in both treatment groups, the maximum effect was not maintained over 24 hours in the II group. Therefore, the time-related effects of ACE inhibition on BP in this 2K-1C model of hypertension seem to be quite different from their effects on BP in spontaneously hypertensive rats. In this latter model, it has been repeatedly shown, with casual indirect measurements, that BP remained lowered for days and weeks after cessation of therapy, whereas the biochemical effects of ACE inhibition disappeared rapidly. This is certainly not the situation in 2K-1C rats, in which BP changes in parallel to the degree of ACE inhibition, which in turn is dependent on the circulating levels of the drug, as shown in normotensive volunteers and hypertensive patients. Mulvany et al. have already reported that the persistent effect of ACE inhibitor therapy seen in spontaneously hypertensive rats is not a general feature of genetic hypertension in rats. In Milan hypertensive rats, no persistent BP effect of perindopril was observed when treatment was stopped, even in the presence of a regression of the vascular structure lesions. The prolonged duration of the BP effects of ACE inhibition in experimental hypertension appears to be dependent on the model and not to constitute a general feature of ACE inhibition. The clinical and biochemical observations made in humans with enalapril are indeed similar to our results in 2K-1C hypertensive rats.

Both continuous and intermittent ACE inhibition were slightly less effective at day 30 than at day 1. Hypertension may become more resistant with time, as indicated by the increase in BP of the C group, or biochemical changes may limit the antihypertensive efficacy of ACE inhibitors. A time-dependent rise in ACE activity was observed in the untreated hypertensive rats and is likely to be more marked in ACE inhibitor–treated rats through an induction of the converting enzyme, which cannot be quantified in the presence of the inhibitor. In addition, PRC was increased in both treated groups, which was probably secondary to the initial fall in Ang II, and the reactive rise in renin may play a role in limiting the hypotensive effect.

Both treatments significantly decreased HR, which might be the consequence of a reinforcement of vagal tone by ACE inhibition or an indirect sign of improved cardiac function by comparison with untreated hypertensive rats with an increased heart weight.

The consequences of intermittent and continuous ACE inhibition and BP reduction were investigated on the reduction in ventricular hypertrophy (beneficial effect) and the atrophy of the clipped kidney (detrimental effect). The intermittent control of BP was almost as effective as the continuous control on the regression of cardiac hypertrophy. Both modes of ACE inhibition...
were statistically equivalent and effective in reducing cardiac hypertrophy, although there was no significant trend for the intermittent inhibition to be less effective. The regression of left ventricular hypertrophy induced by converting enzyme inhibition in rats may not be exclusively due to the fall in BP and the decrease in afterload.**39,40** Linz et al.**41** have reported that a non-antihypertensive dose of an ACE inhibitor (ramipril, 10 μg/kg once a day) was as effective on cardiac hypertrophy of rats with aortic banding as an antihypertensive dose (1 mg/kg once a day). These authors suggest that the inhibition of a cardiac RAS may explain this dissociation between BP levels and cardiac hypertrophy during the administration of a low dose of ramipril. In our experiments, performed on renovascular rats, no dissociation between BP and heart weight was found. The precise measurement of BP by a 24-hour recording has, on the contrary, allowed us to find highly significant correlations between heart weight and the sum of 24-hour BP values within each group. As shown in Fig 3, there is a trend for intermittent therapy to be less effective than continuous therapy, even though this did not reach statistical significance.

The results of the present study on renovascular experimental hypertension indicate that it is difficult to balance good control of BP, regression of left ventricular hypertrophy, and preservation of adequate renal function of a stenotic kidney after ACE inhibition. If applied to patients with renovascular hypertension, the results suggest that the choice between medical treatment or repair of the stenotic renal artery**42** is a balance between the specific risks of the two therapeutic approaches.

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