Angiotensin II Type 1 Receptor Blockade Prevents Lethal Malignant Hypertension
Relation to Kidney Inflammation

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Background—Angiotensin II is elevated in malignant hypertension. We tested the hypothesis that angiotensin II type 1 receptor blockade can prevent the development of malignant hypertension even in the absence of a blood pressure–lowering effect.

Methods and Results—Two-kidney, 1-clip rats were followed up for 28 days; blood pressure was measured by tail-cuff plethysmography and intra-arterially. After a 2-week run-in phase, rats received valsartan at a dose of 0.3 (n=14) or 3 (n=12) mg · kg⁻¹ · d⁻¹ or solvent (n=27). Only the higher dose of valsartan, but not the lower dose, decreased blood pressure. Both doses of valsartan prevented the development of lethal malignant hypertension. Twenty of 27 solvent-treated renovascular hypertensive rats died, but only 3 of 14 rats treated with the low dose and 1 of 12 rats treated with the high dose of valsartan died. Histological signs of malignant nephrosclerosis were found in all rats examined that had died throughout the study and in 6 of 7 surviving solvent-treated renovascular hypertensive animals. Increased expression of monocyte chemoattractant protein-1 and prominent interstitial influx of macrophages occurred in the nonclipped kidneys exposed to high pressure in solvent-treated rats. These alterations were prevented by valsartan at both doses, irrespective of blood pressure effects.

Conclusions—Angiotensin II type 1 receptor blockade by valsartan prevents lethal malignant hypertension independently of blood pressure. The results suggest that reduction of angiotensin-induced inflammation in the kidney may contribute to the protective effects of valsartan. (Circulation. 2001;104:1436-1440.)

Key Words: angiotensin ■ hypertension ■ kidney ■ leukocytes ■ survival

Malignant hypertension is characterized by rapidly progressive target-organ injury, often out of proportion to the level of blood pressure itself. Injury to the kidney is an important feature of malignant hypertension. Moreover, the kidney plays a key role in the development of malignant hypertension: salt wasting and alterations in intrarenal hemodynamics are hallmarks of early malignant nephrosclerosis.¹ ²

Recent studies have reinforced the notion that angiotensin (Ang) II plays an important role in the development of malignant hypertension. Using different transgenic rat models characterized by overexpression of genes of the renin-angiotensin system, Montgomery et al³ and Mervaala et al⁴ ⁵ showed that blockade of Ang II prevented the development of malignant hypertension in these models. These authors provided some evidence that inhibition of converting enzyme³ and renin⁴ were effective even if blood pressure was not lowered. Blood pressure, however, was assessed only by the tail-cuff measurements in anesthetized animals.

We tested the hypothesis that inhibition of the action of Ang II on its type 1 receptor (AT₁)⁶ prevents the occurrence of lethal malignant hypertension independently of blood pressure lowering. Blood pressure was measured intra-arterially. We studied the occurrence of malignant nephrosclerosis in the 2-kidney, 1-clip (2K1C) model of renovascular hypertension, which is comparable to renovascular hypertension in humans. Infiltration of macrophages, which occurs in the kidney exposed to high blood pressure,⁷ ⁸ was investigated as a potential nonhemodynamic mechanism.

Methods

Renovascular Hypertension

Rats were housed in a room maintained at 22±2°C, exposed to a 12-hour dark/light cycle. The animals were allowed unlimited access to chow (No. 1320, Altromin) and tap water. All procedures performed on animals were done in accordance with NIH guidelines...
and were approved by the local government authorities (Regierung von Mittelfranken, AZ No. 621-2531.3-10/94).

2K1C renovascular hypertension was induced in male SpragueDawley rats (Charles River, Sulzfeld, Germany) weighing 150 to 170 g by placing a silastic clip of 0.2-mm ID around the left renal artery through a flank incision under anesthesia with ketamine/xylazine as previously described.7 Control animals underwent sham operation without placement of the clip. Weight and systolic blood pressure (by tail-cuff plethysmography7) were measured weekly.

**Treatment**

After 2 weeks, only animals with systolic blood pressure >150 mm Hg, which was achieved in 80% to 90% of all renal-clip animals, were included in the 2K1C groups. Rats were treated with 2 doses of the AT1 antagonist (AT1 RA) valsartan or placebo. Twelve 2K1C rats and 5 sham-operated rats received 3 mg·kg\(^{-1}\)·d\(^{-1}\) AT1 RA, 14 2K1C rats received 0.3 mg·kg\(^{-1}\)·d\(^{-1}\) valsartan, and 27 2K1C rats and 10 sham-operated rats received solvent. Osmotic minipumps (Alzet model 2002, Alza) that delivered 0.5 μL/h for 14 days were implanted in the peritoneal cavity through a small midline incision under ether anesthesia. Valsartan (55.8 mg/mL for the higher dose and 5.58 mg/mL for the lower dose) was dissolved in 0.46 mol/L KOH (pH 7.2). Animals were followed up for 14 days.

To obtain better measurements of blood pressure during treatment, we performed an additional study. Two weeks after clipping, 6 2K1C rats received the high dose and 6 2K1C animals were treated with the low dose of AT1 RA, and 8 2K1C and 5 sham-operated animals received solvent. After 9 days of treatment, all animals were instrumented with femoral artery catheters for intra-arterial blood pressure measurements under ether anesthesia as described previously.8 Measurements were performed on the same day 4 hours after termination of anesthesia via transducers connected to a polygraph (Hellige).

After the organs had been weighed, kidneys were decapsulated. Part of each kidney was immediately snap-frozen on liquid nitrogen for protein and RNA extraction, and a second part was put in glacial acetic acid) for fixation.

**Immunohistochemistry of Kidney Sections**

After overnight fixation in methyl–Carnoy solution, tissues were dehydrated and embedded in paraffin. Sections (2 μm) were cut with a Leitz SM 2000 R microtome (Leica Instruments). After deparaffinization and blockade of endogenous peroxidase activity, the immunohistochemical detection of macrophages (monoclonal ED-1 antibody, Biozol), macrophage chemoattractant protein-1 (MCP-1; polyclonal rabbit antiserum kindly provided by Dr T. Yoshimura, Biozol), macrophage chemoattractant protein-1 (MCP-1; polyclonal rabbit antiserum kindly provided by Dr T. Yoshimura, Biozol) was performed as previously described.8 The Vectastain DAB kit (Vector Laboratory) was used as a chromogen. Each slide was counterstained with hematoxylin. Renal interstitial macrophages (monoclonal ED-1 antibody, Biozol) was performed as previously described.9 The Vectastain DAB kit (Vector Laboratory) was used as a chromogen. Each slide was counterstained with hematoxylin. Renal interstitial macrophages were counted as described previously in 30 medium-power (magnification ×250) cortical views per section and expressed as cells/mm\(^2\). Interstitial collagen I was quantified in 30 medium-power views by means of an 11×11-point grid. The percentage of grid points corresponding to a stained area was calculated.

**Northern and Western Blot for MCP-1 mRNA and Protein**

RNA and protein were extracted from kidney cortical tissue by use of TRI reagent (MRC Inc). Northern blot analysis was performed with full-length rat MCP-1 cDNA as described previously.8 Western blot was performed after proteins had been separated with a denaturing 10% to 18% SDS–polyacrylamide gradient gel in the buffer system described by Schägger and von Jagow.10 The membrane was incubated in a 1:1000 dilution of an MCP-1 antibody and a peroxidase-conjugated secondary antibody, followed by luminescence immunodetection (ECL, Amersham). The x-ray films used for detection were then analyzed by densitometry.

**Results**

On the basis of weight measurements, rats treated with low-dose AT1 RA received a mean dose of 0.32 mg·kg\(^{-1}\)·d\(^{-1}\) valsartan (SD 0.44 mg·kg\(^{-1}\)·d\(^{-1}\), n=12) or valsartan (SD 0.05 mg·kg\(^{-1}\)·d\(^{-1}\), n=14) for 14 days. Drug therapy began 14 days after renal artery clipping. A significant differences between AT1 RA–treated and solvent-treated 2K1C rats.

**Statistical Analysis**

Two-way ANOVA, followed by Bonferroni’s post hoc test with adjustment for multiple comparison, was used to test the significance of differences between groups. A value of P<0.05 was considered significant. The procedures were carried out with Statistica software (StatSoft). Values are displayed as mean±SEM unless indicated otherwise.

**Figure 1.** Systolic blood pressure (A) and survival (B) of sham-operated (n=15) and renovascular hypertensive rats treated with solvent (n=27) or valsartan (AT1 RA) in high dose (3 mg·kg\(^{-1}\)·d\(^{-1}\), n=12) or low dose (0.3 mg·kg\(^{-1}\)·d\(^{-1}\), n=14) for 14 days. Death was usually preceded by 3 to 5 days of weight loss. Both high- and low-dose regimens of AT1 RA prevented mortality (Figure 1B).
AT1 RA had no effect on blood pressure or organ weight (data not shown).

Screening by light microscopy of routine kidney sections of all surviving animals and 11 that had died for signs of malignant hypertension (fibrinoid necrosis and/ or onion-shaped vascular cell proliferative lesions) revealed the presence of these changes in all animals that had died throughout the study, in all but 1 (6 of 7) surviving solvent-treated 2K1C rats, and in 4 of 11 low-dose AT1 RA-treated 2K1C but not in any sham-operated or high-dose AT1 RA–treated rat (see Figure 3 for examples).

Extracellular matrix accumulation in the nonclipped kidney exposed to high blood pressure was demonstrated by quantification of interstitial collagen I in 2K1C rats (16.7 ± 1.7% versus 6.4 ± 0.9% of cortical area in sham-operated rats, \( P < 0.05 \)). Collagen I was decreased by low-dose valsartan (12.1 ± 2.3%, \( P < 0.05 \) versus untreated 2K1C), and to an even greater degree by high-dose valsartan (8.3 ± 2.2%, \( P < 0.01 \) versus untreated 2K1C, \( P < 0.05 \) versus low-dose valsartan).

There were more interstitial macrophages in the nonclipped kidney exposed to high blood pressure of 2K1C animals than in normal kidneys (Figure 2B). High- and low-dose regimens of AT1 RA decreased macrophage infiltration to the same degree (Figure 2B). MCP-1 mRNA was increased significantly, to 172 ± 8% of control levels in the nonclipped kidneys of 2K1C (Figure 4A). Valsartan blunted the increase of MCP-1 mRNA (101 ± 20% of control levels at the low dose and 72 ± 7% at the high dose, \( P < 0.05 \)). The elevation of MCP-1 protein (206 ± 3% of control levels, \( P < 0.05 \)) was also blunted by both doses of AT1 RA (95 ± 6% of control levels at the low dose and 86 ± 9% at the high dose, \( P < 0.05 \), Figure 4B). By immunohistochemistry, MCP-1 protein was detected in vascular smooth muscle cells, interstitial cells, glomerular epithelial cells, macrophages, and occasionally but rarely in tubular cells in the nonclipped

**Figure 2.** Arterial blood pressure (A) and renal macrophage infiltration (B). Mean arterial pressure was measured via femoral artery catheters in conscious rats (n=5 per group) 23 days after renal artery clipping after 9 days of drug treatment. Interstitial macrophage influx in nonclipped right kidney exposed to high blood pressure was counted after immunostaining for macrophage marker ED-1. See Figure 1B for numbers of animals per group. 2K1C indicates renovascular hypertensive rats; AT1RA, valsartan 0.3 (low dose) or 3 (high dose) mg · kg\(^{-1} \) · d\(^{-1} \).

\*\( P < 0.05 \) vs sham-operated animals; § \( P < 0.05 \) vs untreated (solvent) 2K1C.

**Figure 3.** Examples of vascular lesions in 2K1C renovascular hypertensive rats after periodic acid–Schiff staining. A to C, Right kidneys; D and E, heart. A, Bar=100 μm; same magnification for all panels. Arrows point to normal vessels in A (interlobular artery with branching afferent arteriole) and D (coronary vessel), sections from sham-operated rats. B, Fibrinoid necrotic lesion (arrow) surrounded by cells in right, nonclipped kidney of 2K1C rats exposed to hypertension. E, Arrows point to similar vascular lesions in heart of 2K1C rat. C, Section from right, nonclipped kidney of 2K1C rat exposed to hypertension. Black arrowhead indicates a vascular lesion with hyalinosis, necrosis, and occlusion; white arrowhead, hyalinosis of entire glomerular tuft.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Body Weight, g</th>
<th>Left Kidney/BODY Wt Ratio, mg/kg</th>
<th>Right Kidney/BODY Wt Ratio, mg/kg</th>
<th>Heart/BODY Wt Ratio, mg/kg</th>
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<tbody>
<tr>
<td>Sham-operated rats (n=15)</td>
<td>320 ± 4</td>
<td>3.50 ± 0.02</td>
<td>3.53 ± 0.03</td>
<td>3.40 ± 0.05</td>
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<tr>
<td>Untreated 2K1C rats (n=7)</td>
<td>166 ± 11*</td>
<td>3.49 ± 0.11</td>
<td>7.47 ± 0.51</td>
<td>6.57 ± 0.33*</td>
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<td>2K1C/AT1RA low dose (n=11)</td>
<td>228 ± 11*†</td>
<td>2.94 ± 0.27†</td>
<td>5.31 ± 0.28†</td>
<td>5.35 ± 0.22†</td>
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<tr>
<td>2K1C/AT1RA high dose (n=11)</td>
<td>263 ± 9††</td>
<td>2.43 ± 0.22††</td>
<td>5.21 ± 0.22††</td>
<td>4.56 ± 0.29††</td>
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\* \( P < 0.05 \) vs sham operation; †significant differences in AT1RA-treated vs untreated 2K1C; ‡ \( P < 0.05 \) between the high and the low dose of AT1RA.
kidney of untreated 2K1C rats, as we described in more detail recently.8 In valsartan-treated 2K1C rats, MCP-1 staining was restricted to vascular smooth muscle cells, as in sham-operated animals.

Discussion

Our results show that blockade of the AT1 receptor by valsartan prevents the development of lethal malignant hypertension in renovascular hypertensive rats even if blood pressure is not decreased. Furthermore, the data suggest that AT1-mediated nonpressor, proinflammatory effects, ie, induction of the chemokine MCP-1 and macrophage infiltration, may contribute to the development of malignant hypertension.

Our observation that AT1 blockade prevented malignant hypertension regardless of its effect on blood pressure confirms and extends recent reports in other models of malignant hypertension in the rat.3–5 Montgomery et al11 showed that ACE inhibition lowered the frequency of malignant hypertension in Ren2-transgenic hypertensive rats. These authors used a dose of ACE inhibition that did not lower systolic blood pressure as measured by a tail-cuff method under anesthesia, but they did not perform intra-arterial recordings.3 Mervaala and coworkers4,5 used a new transgenic rat model with extreme overexpression of the human renin and angiotensinogen genes, which leads to early mortality in all rats.4 In these animals, mortality could be prevented if blood pressure was lowered with blockers of the renin-angiotensin system but not with a conventional triple therapy, despite comparable antihypertensive efficacy, as judged from tail-cuff measurements.5

Unlike these transgenic models of malignant hypertension,3–5 which are characterized by an extreme overexpression of genes of the renin-angiotensin system and are therefore expected to be dependent on Ang II, renovascular hypertension is an important cause of high blood pressure in human patients. The prevalence of renovascular hypertension among all hypertensive patients is subject to debate (for review see Conlon et al11) and appears to be lower than previously thought.12 There is little doubt, however, that renovascular hypertension is associated with a higher cardiovascular morbidity and mortality than essential hypertension.13 Rats with unilateral renal artery clips develop renovascular hypertension and malignant hypertension in a predictable manner, depending on the size of the animal and the ID of the clip.1 Death from malignant hypertension is usually preceded by several days of volume and weight loss.1

Our results demonstrate an important role of the AT1 receptor for the development of malignant hypertension. The AT1 receptor antagonist valsartan prevented lethal malignant hypertension even when blood pressure was not affected. To avoid a possible selection bias due to mortality of the animals with the highest blood pressure levels, we measured intra-arterial blood pressure in a second group of animals followed up for a somewhat shorter time. In addition to our blood pressure measurements, the notion that 0.3 mg·kg$^{-1}$·d$^{-1}$ valsartan has no important effect on blood pressure is also supported by previous reports from other laboratories,13,14 including telemetric measurements performed by Webb et al.14 These authors reported only minute effects of a somewhat higher dose of valsartan, 0.5 mg·kg$^{-1}$·d$^{-1}$, on blood pressure.14 In the absence of telemetric measurements, we cannot exclude such minute effects in our study, but we consider it highly unlikely that the massive effects of valsartan on survival can be explained solely by lowering of the mean arterial blood pressure by some 5 mm Hg. Interestingly, blockade of the renin-angiotensin system was reported to improve survival in scleroderma renal crisis,15 another form of thrombotic microangiopathy in the kidney. Thus, our data may have implications for human renal diseases beyond renovascular hypertension.

We do not try, however, to dispute the importance of high blood pressure for target organ damage. Some aspects of organ protection by valsartan were clearly dependent on blood pressure itself: both the organ weights and the measurement of renal interstitial collagen I indicate that the higher dose of valsartan, which lowered blood pressure, caused a greater amelioration of heart hypertrophy and nephrosclerosis. Unfortunately, the higher dose of valsartan also induced a more pronounced weight loss of the poststenotic left kidney, reminiscent of previous reports on the effects of ACE inhibition on the poststenotic kidney.16,17

Which factor(s) might explain the blood pressure–independent effect of valsartan on survival? We think that our data support the notion that AT1-mediated proinflammatory effects are important for the development of lethal malignant nephrosclerosis. We and others have previously shown that a marked infiltration of macrophages into the kidney occurs in angiotensin-dependent forms of hypertension, including Ang II infusion,18,19 renovascular hypertension,7,8,20,21 and overexpression of genes of the renin-angiotensin system in transgenic rats.4,8 Interstitial macrophage infiltration was decreased by valsartan regardless of whether or not blood pressure was affected.

The influx of macrophages may be due to AT1-mediated induction of the chemokine MCP-1. Ang II has been shown to induce MCP-1 expression via AT1 in vascular smooth mus-
served that MCP-1 RNA and protein induction were blunted by valsartan independently of blood pressure, similar to its effects on macrophages. The precise role of MCP-1 for macrophage infiltration remains unclear because other chemokines or adhesion molecules might also contribute and because AT1 may activate macrophages directly. Results obtained with blocking antibodies against MCP-1 in glomerulonephritis, however, support the notion that MCP-1 plays a pivotal role for macrophage infiltration in the kidney. Moreover, the CCR2 receptor for MCP-1 is necessary for macrophage infiltration in aortic tissue in atherosclerosis and hypertension.

In summary, we propose that the systemic activation of the renin-angiotensin system caused by obstructing one renal artery not only elevates blood pressure but also induces an inflammatory response in the nonclipped kidney. This inflammatory response is mediated via the AT1 receptor and is at least partially independent of blood pressure. AT1-mediated induction of the chemokine MCP-1 attracts macrophages, which in turn contribute to the development of renal tissue injury and malignant hypertension. Even a nonhypotensive dose of an AT1 antagonist, such as valsartan, can prevent this potentially lethal complication of hypertension.

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