Effect of Angiotensin II Blockade on Renal Injury in Mineralocorticoid-Salt Hypertension

Mitra Soroshian, Jean L. Olson, Timothy W. Meyer

Abstract—Kidney function and structure were compared in control rats (group 1) and in 3 groups of rats made hypertensive by administration of aldosterone and saline for 8 weeks (groups 2, 3, and 4). Group 2 rats received only aldosterone and saline, while group 3 also received losartan and group 4 also received enalapril. Rats in all groups were subjected to uninephrectomy before beginning the experiment. Hypertension and proteinuria in rats given aldosterone and saline were not affected by losartan or enalapril (8-week values for blood pressure in mm Hg: 135±3 group 1, 193±4 group 2, 189±4 group 3, 189±5 group 4; P<0.05 groups 2, 3, and 4 versus 1; 8-week values for proteinuria in mg/d: 44±8 group 1, 278±34 group 2, 267±37 group 3, 289±36 group 4; P<0.05 groups 2, 3, and 4 versus 1). Vascular, glomerular, and tubulointerstitial injury accompanied hypertension and proteinuria at 8 weeks. Losartan and enalapril did not prevent vascular injury, which was characterized by thickening of arterial and arteriolar walls and by fibrinoid necrosis and thrombotic microangiopathy. Likewise, losartan and enalapril did not reduce the prevalence of glomerular segmental sclerosis (1±1% group 1, 10±2% group 2, 11±2% group 3, 13±2% group 4; P<0.05 groups 2, 3, and 4 versus 1) or limit tubulointerstitial injury as reflected by the volume fraction of the cortical interstitium (15±1% group 1, 20±1% group 2, 21±1% group 3, 21±1% group 4; P<0.05 groups 2, 3, and 4 versus 1). These findings suggest that local angiotensin II activity does not contribute to the development of renal injury in mineralocorticoid-salt hypertension. (Hypertension. 2000;36:569-574.)

Key Words: hypertension, experimental mineralocorticoids angiotensin glomerular filtration rate interstitium

Experimental hypertension is usually accompanied by renal injury. The pattern of injury varies somewhat in different hypertensive models. In general, however, arterial wall changes are accompanied by glomerular sclerosis, tubular atrophy, and interstitial fibrosis. Recent studies have shown that angiotensin II (Ang II) can contribute to injury at each of these sites within the kidney. The mechanism(s) by which Ang II causes renal injury, however, remains controversial. A number of studies have identified local contributions of Ang II to renal injury that appear to be independent of the effects of Ang II on blood pressure.2-5 In particular, some studies have suggested that when renal tubules are injured, increased local Ang II production contributes to the development of interstitial fibrosis. Other studies have suggested that when blood pressure is elevated, local vascular and glomerular Ang II production contributes to vascular injury and glomerular sclerosis. Determining the extent to which direct, local actions of Ang II cause renal injury associated with hypertension, however, presents a problem. Drugs that reduce Ang II activity usually both lower blood pressure and limit injury. This dual effect makes it hard to separate the hypertensive action of Ang II, with its complex but indirect damage, from the direct fibroproliferative action of Ang II on renal cells. These 2 pathways to injury can be discriminated, however, in a model in which Ang II blockade does not lower blood pressure. The present study therefore examined the effect of Ang II blocking agents on renal injury in rats with mineralocorticoid-salt hypertension. We found that neither converting enzyme inhibition nor Ang II receptor blockade limited injury at any site within the kidney while blood pressure remained elevated.

Methods

Male Munich-Wistar rats weighing 272 to 295 g were subjected to right uninephrectomy under anesthesia provided by methohexital 50 mg/kg IP. One week later osmotic mini-pumps (model 2 ML4; Alza) were installed subcutaneously with the use of the same anesthetic. Group 1 (n=8) received saline via the pumps, while the remainder of the rats received d-aldosterone at a rate of 45 μg/kg per day. All rats were given a solution of 1% NaCl and 0.3% KCl in water to drink. Two weeks were allowed after installation of the pumps for rats receiving aldosterone to become hypertensive. These rats were then divided into 3 groups. Group 2 (n=10) continued to receive no treatment other than aldosterone, while group 3 (n=11) received losartan 180 mg/L in the drinking solution and group 4 (n=10) received enalapril 150 mg/L in the drinking solution. Alza pumps were replaced at 4 weeks so that the total duration of pump treatment was 8 weeks and the total duration of treatment with losartan or enalapril was 12 weeks.
enalapril was 6 weeks. Systolic blood pressure was measured at 2, 4, 6, and 8 weeks by the tail-cuff method. Twenty-four-hour urinary protein excretion was measured at 5 and 8 weeks by the Coomassie blue method.

At 8 weeks, rats were anesthetized with Inactin, 100 mg/kg IP, and subjected to studies of kidney function and structure. Glomerular filtration rate was measured over two 30-minute clearance periods in animals maintained euvolemic by infusion of saline and rat plasma. After the clearance measurements, the effectiveness of Ang II receptor blockade and converting enzyme inhibition was confirmed by assessing the pressor responses to 50-ng bolus infusions of angiotensin I (Ang I) and Ang II, and blood samples were collected for determination of plasma sodium and potassium concentrations. Kidneys were then fixed by retrograde aortic perfusion and weighed. Transverse kidney slices were embedded in paraffin, and sections were stained with hematoxylin and eosin and with the periodic acid–Schiff technique. The prevalences of segmental glomerular lesions and glomerular microaneurysms were determined by examining all glomerular profiles in a single section from each animal (average, 150 ± 5 profiles). Segmental lesions were most often characterized by areas of the tuft showing collapse of the glomerular capillaries, accompanied by hyalinosis and focal adhesion of the tuft to the Bowman’s capsule. In addition, some glomeruli showed prominent distention of capillary loops by proteinaceous material. Microaneurysms were defined by the presence of mesangiosis with extreme dilation of glomerular capillary loops containing fibrin, erythrocytes, and other cellular elements.

Average values for the glomerular tuft volume and the fractional volume of cortical interstitium in each animal were determined as previously described.1 The extent of vascular injury was assessed by examining profiles of the arteries and arterioles in a single kidney section from each animal. Each profile was first categorized according to whether its shortest external diameter measured <50, 50 to 100, or >100 µm. Profiles exhibiting fibroinoid necrosis characterized by infiltration of the wall with amorphous, eosinophilic material or thrombotic microangiopathy characterized by fibrin-platelet thrombi and red cell fragments were further categorized as showing overt wall injury. The prevalence of wall injury was expressed as the percentage of vessel profiles showing these changes. Vascular wall thickness was then measured by a modification of the procedure described by Whitworth et al.1 Wall thickness was measured only in vascular profiles that did not show overt injury. For each profile, the total and luminal areas were measured with a computer-assisted morphometric unit. The ratio of the wall area to luminal area was then calculated as the ratio of the total area minus the luminal area to the luminal area. Vessels that appeared to have been cut very obliquely, as evidenced by a ratio of the longest to shortest external diameter of >5:1, were excluded from this analysis.

ANOVA and Fisher’s probability of least significant difference were used to assess the significance of differences between the groups. Significance was defined as P<0.05, and results are expressed as mean±SE throughout. The animal protocol was approved by the institutional review board.

Results

Systolic blood pressure values in awake rats are depicted in Figure 1. Values in group 1 rats subjected to uninephrectomy and given saline to drink were similar to those obtained in normal rats in our laboratory. Infusion of aldosterone along with provision of saline caused prominent hypertension in groups 2, 3, and 4. The aldosterone-induced increase in blood pressure was not affected by Ang II receptor blockade in group 3 or converting enzyme inhibition in group 4. Protein excretion remained low in group 1 rats, averaging 53±7 mg/d at 5 weeks and 44±8 mg/d at 8 weeks. Sustained hypertension was accompanied by the development of heavy proteinuria in groups 2, 3, and 4. Ang II receptor blockade and converting enzyme inhibition had no effect on protein excretion, which at 5 weeks averaged 207±26 mg/d in group 2, 213±24 mg/d in group 3, and 260±40 mg/d in group 4 and at 8 weeks averaged 278±34 mg/d in group 2, 267±37 mg/d in group 3, and 289±36 mg/d in group 4.

Kidney function studies at 8 weeks are summarized in Table 1. Mineralocorticoid-salt hypertension was associated with impaired growth, so that body weight was less in groups 2, 3, and 4 than in group 1. Values for hematocrit and plasma protein concentration were similar in all 4 groups. The groups receiving aldosterone exhibited a slight increase in plasma sodium concentration without a significant reduction in plasma potassium concentration. Values for mean arterial pressure under anesthesia paralleled values for systolic blood pressure obtained in awake animals. The mean arterial pressure of 110±3 mm Hg in group 1 was similar to the value we have observed in normal rats. Chronic aldosterone infusion increased mean arterial pressure to 148±3 mm Hg in group 2.

### Table 1. Summary of Functional Studies at 8 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, g</th>
<th>Hematocrit</th>
<th>C₆, g/dL</th>
<th>[Na], mmol/L</th>
<th>[K], mmol/L</th>
<th>MAP, mm Hg</th>
<th>GFR, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=7)</td>
<td>340±5</td>
<td>0.46±0.01</td>
<td>5.5±0.1</td>
<td>144±1</td>
<td>3.7±0.1</td>
<td>110±3</td>
<td>2.36±0.13</td>
</tr>
<tr>
<td>Group 2 (n=9)</td>
<td>327±4*</td>
<td>0.46±0.01</td>
<td>5.6±0.1</td>
<td>151±2*</td>
<td>3.5±0.1</td>
<td>148±3*</td>
<td>1.95±0.11*</td>
</tr>
<tr>
<td>Group 3 (n=9)</td>
<td>321±6*</td>
<td>0.44±0.02</td>
<td>5.4±0.1</td>
<td>149±1*</td>
<td>3.3±0.1*</td>
<td>145±2*</td>
<td>1.86±0.12*</td>
</tr>
<tr>
<td>Group 4 (n=7)</td>
<td>315±4*</td>
<td>0.44±0.02</td>
<td>5.5±0.1</td>
<td>148±1*</td>
<td>3.4±0.1</td>
<td>143±2*</td>
<td>1.76±0.12*</td>
</tr>
</tbody>
</table>

C₆ indicates plasma protein concentration; [Na], serum sodium concentration; [K], serum potassium concentration; MAP, mean arterial pressure; and GFR, glomerular filtration rate. Functional studies were not performed in 1 of 8 rats in group 1, 1 of 10 rats in group 2, 2 of 11 rats in group 3, and 3 of 10 rats in group 4.

*P<0.05 vs group 1.
Neither Ang II receptor blockade nor converting enzyme inhibition altered mean arterial pressure, which averaged 145±2 mm Hg in group 3 and 143±2 mm Hg in group 4. The glomerular filtration rate in normotensive group 1 rats averaged 2.36±0.13 mL/min. The average glomerular filtration rate was reduced in each of the hypertensive groups but was not affected by drug treatment, averaging 1.95±0.11 mL/min in group 2, 1.86±0.12 mL/min in group 3, and 1.76±0.12 mL/min in group 4.

Structural findings at 8 weeks are summarized in Table 2 and depicted in Figures 2 through 5. Significant structural abnormalities were not observed in group 1. Mineralocorticoid-salt hypertension, however, was associated with prominent structural changes in groups 2, 3, and 4. Kidney weight was increased in each of these groups. Thickening of the vascular wall was observed at all levels of the arterial tree. Morphometric studies showed an increase in the wall to lumen area ratio that was most prominent in small arteries and arterioles. The diffuse increase in wall thickness was accompanied by focal wall injury. Wall injury was characterized by the appearance of fibrinoid necrosis and thrombotic microangiopathy (Figure 2). Both features of injury were more common in smaller vessels. Neither diffuse wall thickening nor focal wall injury was affected by Ang II receptor blockade in group 3 or converting enzyme inhibition in group 4.

Vascular injury was accompanied by glomerular injury in each of the hypertensive groups. Average glomerular volume was increased from 1.70±0.09×10⁶ μm³ in group 1 rats subjected only to uninephrectomy to 2.23±0.13×10⁶ μm³ in group 2 rats with mineralocorticoid-salt hypertension. Glomerular volume was not affected by Ang II receptor blockade or converting enzyme inhibition. The majority of glomeruli appeared normal, but a significant number exhibited segmental lesions in each of the hypertensive groups. Injured glomeruli most often showed segmental sclerosis characterized by collapse of capillary loops and adhesion of the tuft to Bowman’s capsule (Figure 3). Segmental distention of capillary loops by proteinaceous material associated with decreased glomerular cellularity was also observed (Figure 4). These lesions sometimes involved the majority of the tuft. Visceral epithelial cells showed severe injury accompanied by denudation of the glomerular basement membrane. Some glomerular capillary loops showed fibrin thrombi and fragmented red cells characteristic of thrombotic microangiopathy. Occasional glomeruli contained microaneurysms characterized by mesangiolysis and extreme dilatation of glomerular capillary loops containing fibrin, erythrocytes, and other cellular elements (Figure 5). Microaneurysms often filled Bowman’s space and appeared in many cases to have fibrinoid necrosis with adhesion of the tuft to Bowman’s capsule (hematoxylin and eosin, magnification ×300).

### Table 2. Summary of Morphological Studies at 8 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney Weight, g</th>
<th>Kidney Weight/Body Weight, mg/g</th>
<th>Wall to Lumen Ratio</th>
<th>% Wall Injury</th>
<th>Glomeruli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;50 μm</td>
<td>50–100 μm</td>
<td>&gt;100 μm</td>
</tr>
<tr>
<td>Group 1</td>
<td>2.2±0.1</td>
<td>6.5±0.2</td>
<td>0.51±0.02</td>
<td>0.38±0.03</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>Group 2</td>
<td>3.1±0.1*</td>
<td>9.5±0.2*</td>
<td>1.20±0.10*</td>
<td>0.63±0.07*</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>Group 3</td>
<td>3.1±0.1*</td>
<td>9.8±0.2*</td>
<td>1.34±0.12*</td>
<td>0.74±0.12*</td>
<td>0.44±0.05*</td>
</tr>
<tr>
<td>Group 4</td>
<td>3.1±0.1*</td>
<td>9.7±0.3*</td>
<td>1.41±0.12*</td>
<td>0.72±0.04*</td>
<td>0.51±0.04*</td>
</tr>
</tbody>
</table>

Figure 2. Light micrograph of 2 arterioles, one showing marked fibrinoid necrosis (arrow) and the other showing thrombotic microangiopathy characterized by a fibrin–platelet thrombus containing fragmented red cells (hematoxylin and eosin, magnification ×300).

Figure 3. Micrograph of glomerulus showing segmental sclerosis with adhesion of the tuft to Bowman’s capsule (hematoxylin and eosin, magnification ×300).
ruptured. Other glomeruli showed an appearance suggestive of ischemia characterized by wrinkling of capillary loops and collapse of capillary lumina. Glomerular injury, like vascular injury, was unaffected by Ang II receptor blockade and converting enzyme inhibition.

Glomerular and vascular injury in hypertensive rats was accompanied by patchy tubulointerstitial injury characterized by tubule epithelial cell damage, a chronic inflammatory infiltrate, and interstitial edema and fibrosis. Scattered tubule casts were also observed. The development of tubulointerstitial injury was reflected by an increase in the interstitial volume fraction from 15 ± 1% in normotensive group 1 rats to 20 ± 1% in group 2 rats with mineralocorticoid-salt hypertension. This change, like the other features of injury examined, was not affected by Ang II receptor blockade or converting enzyme inhibition.

The effectiveness of Ang II receptor blockade and converting enzyme inhibition was confirmed by assessing the pressor responses to intravenous bolus infusions of Ang I and Ang II in a subset of animals (n = 5 to 9 in each group). The pressor response to 50 ng of Ang I was 49 ± 5 mm Hg in group 1, 45 ± 3 mm Hg in group 2, 4 ± 2 mm Hg in group 3 (P < 0.05 versus group 1 and 2), and 5 ± 2 mm Hg in group 4 (P < 0.05 versus group 1 and 2). The pressor response to 50 ng of Ang II was 52 ± 3 mm Hg in group 1, 41 ± 3 mm Hg in group 2, 4 ± 1 in group 3 (P < 0.05 versus groups 1, 2 and 4), and 56 ± 5 mm Hg in group 4. Thus, both enalapril and losartan reduced the pressor response to Ang I, while losartan reduced the pressor response to Ang II.

**Discussion**

The goal of this study was to determine whether local Ang II action is necessary in the development of hypertensive renal injury. Studies were performed in rats with mineralocorticoid-salt hypertension so that the contributions to injury of local Ang II action and blood pressure could be distinguished. Previous studies have shown that rats with mineralocorticoid-salt hypertension develop progressive vascular, glomerular, and tubulointerstitial disease.

Previous studies have also shown that blood pressure in these animals is not reduced by converting enzyme inhibition or Ang II receptor blockade. These findings were confirmed in the present study, making it possible to assess the contribution of Ang II to injury at different sites within the kidney.

The first finding of this study was that agents that reduce Ang II activity did not limit vascular injury in rats with mineralocorticoid-salt hypertension. As expected, hypertension was accompanied by an increase in the wall to lumen area ratio throughout the renal arterial tree. Two lines of evidence have suggested that Ang II can contribute to this structural change. First, Ang II has been shown to cause vascular smooth muscle cells to grow and produce matrix components. Second, mechanical strain has been shown to potentiate the local effects of Ang II produced within the vascular wall. These findings suggest that hypertension can promote vessel wall thickening by increasing local Ang II activity.

We found, however, that losartan and enalapril did not reduce wall to lumen ratios in rats with mineralocorticoid-salt hypertension. It thus appears that a strain-induced increase in Ang II activity does not contribute significantly to vessel wall thickening in this setting. In addition to generalized arterial wall thickening, hypertension was accompanied by the appearance of fibrinoid necrosis and thrombotic microangiopathy. The pathogenesis of these forms of vascular injury, which are commonly observed in severe hypertension, is not fully understood. Ang II has been considered a potential contributor to fibrinoid necrosis, however, because it increases endothelial permeability to plasma proteins. Ang II has likewise been identified as a potential contributor to thrombotic injury because it increases endothelial expression of plasminogen activator inhibitor.
The second finding of this study was that agents that reduce Ang II activity did not prevent glomerular injury in mineralocorticoid-salt hypertension. The development of glomerular injury was manifested by increasing proteinuria, which was not affected by treatment with losartan or enalapril. Morphological examination revealed glomerular segmental sclerosis similar to that seen in other models of hypertension. Studies analogous to those performed in arteries and smooth muscle cells have suggested that local Ang II action can contribute to this type of injury. Like smooth muscle cells, mesangial cells not only contract but grow and produce matrix components when exposed to Ang II. Studies in rats subjected to renal ablation have identified increased renin and angiotensinogen production in remnant glomeruli. We found, however, that losartan and enalapril did not reduce the extent of glomerular sclerosis in mineralocorticoid-salt hypertension. Losartan and enalapril also did not reduce microaneurysm formation, which has been identified as a possible precursor to glomerular sclerosis in mineralocorticoid-salt hypertension. The finding that losartan and enalapril did not affect these forms of glomerular injury suggests that local Ang II production was not important in their pathogenesis. In accord with previous reports, rats with mineralocorticoid-salt hypertension had larger kidneys and larger glomeruli than rats subjected to uninephrectomy alone. These interesting hypertrophic changes have not been associated with an increase in glomerular filtration rate, and their cause remains obscure. The present study found that glomerular size and kidney size were not effected by losartan or enalapril. Evidence from other disease models suggests that the increase in glomerular volume may have contributed to the development of glomerular sclerosis.

Finally, the present study found that agents that reduce Ang II activity did not prevent tubulointerstitial injury in mineralocorticoid-salt hypertension. The pathogenesis of tubulointerstitial injury in this and other models of experimental hypertension remains uncertain. It has been suggested, however, that transmission of increased arterial pressure to interstitial capillaries may cause capillary injury resulting in tubular ischemia. There is also strong evidence that absorption of filtered proteins can cause tubule injury. Tubule injury, whether caused by ischemia or excessive protein absorption, is thought in turn to precipitate interstitial fibrosis. Recent studies have shown that local production of Ang II can contribute to interstitial fibrosis in damaged kidneys. Profibrotic actions of Ang II identified in these studies include stimulation of transforming growth factor-β expression, mononuclear cell infiltration, and matrix production. The contribution of local Ang II to interstitial fibrosis is difficult to identify in disease models in which Ang II blockade prevents hypertension and/or reduces proteinuria. The strongest evidence that Ang II promotes interstitial fibrosis has thus come from studies showing a beneficial effect of Ang II blockade in models that develop fibrosis in the absence of hypertension and proteinuria, including rats with cyclosporine nephrotoxicity and ureteral obstruction.

The present study, in contrast, examined the effect of Ang II blockade in rats that had severe hypertension and heavy proteinuria. The results showed that local Ang II production was not a major contributor to interstitial injury in this setting.

The present study was prompted in part by a discrepancy in previously reported results. Dworkin et al found that converting enzyme inhibition did not reduce blood pressure or proteinuria in rats with mineralocorticoid-salt hypertension. They concluded that failure to reduce proteinuria represented failure to prevent injury and did not examine renal tissue. In contrast, Kim et al found that both converting enzyme inhibition and Ang II receptor blockade limited proteinuria in mineralocorticoid-salt hypertension. Lower protein excretion was accompanied by reduced renal expression of matrix constituents. In a follow-up study, Wada et al again found that converting enzyme inhibition and Ang II receptor blockade limited proteinuria. Semi quantitative scoring of 12 structural parameters revealed significant, albeit incomplete, protection against injury. Converting enzyme inhibition significantly reduced the score for 1 structural parameter, while Ang II receptor blockade significantly reduced the score for 5.

The dose of enalapril used by Dworkin et al and by us exceeded that used by Kim et al and Wada et al. It should be also be noted that Dworkin et al observed no protection against injury when enalapril treatment was begun immediately after initiation of mineralocorticoid treatment. It is unclear why Kim et al and Wada et al saw some protection against injury, while we and Dworkin et al saw none. One possible explanation is that the rats studied by Kim et al and Wada et al had entered an accelerated phase of hypertension in which renin released in response to initial vascular injury was contributing to further renal damage. The observation of a late rise in plasma renin activity by Wada et al is consistent with this possibility. In this regard, it is important to emphasize that the present study should not be taken to suggest that local Ang II production cannot cause renal injury. There is strong evidence that the presence of Ang II speeds the operation of several injurious processes in vitro and in vivo. Blood pressure reduction with Ang II blockade has been shown to prevent injury more effectively than blood pressure reduction without Ang II blockade in several disease models. The hypothesis that increased intrarenal Ang II activity promotes injury when blood pressure is reduced without Ang II blockade provides an attractive explanation for these findings. The present study does show, however, that Ang II is not an essential participant in renal vascular or glomerular injury associated with hypertension and proteinuria. Likewise, the present study shows that interstitial injury can develop independent of Ang II activity in this setting.

Acknowledgments

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References


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