Angiotensin-Converting Enzyme Inhibition Reduces the Effect of Bromoethylamine-Induced Papillary Necrosis and Renal Fibrosis

SANDRA L. GARBER,*‡ YELENA MIROCHNIK,† SAPAN S. DESAI,‡ JOSE A. L. ARRUDA,*§ and GEORGE DUNEA*†

*Section of Nephrology, Cook County Hospital, †Hektoen Institute, ‡University of Illinois at Chicago, and §the Veterans Administration West Side Medical Center, Chicago, Illinois.

Abstract. Rats injected with a single, 50-mg dose of bromoethylamine (BEA) developed papillary necrosis accompanied by severe interstitial fibrosis. At 1 mo, the creatinine clearance decreased (control 0.66 versus BEA 0.33 ml/min per 100 g body wt, \(P = 0.02\)), and the urine albumin-to-creatinine ratio increased markedly (control 0.19 versus BEA 0.51, \(P = 0.02\)). In a group of animals given the angiotensin-converting enzyme inhibitor enalapril (Enal; 100 mg/L) in their drinking water for 4 wk, beginning 1 wk before BEA injection, creatinine clearance improved significantly (BEA 0.33 versus Enal + BEA 0.52 ml/min per 100 g body wt, \(P = 0.01\)) and albumin excretion fell to zero. Histologic examination revealed an 88% decrease in the area of papillary necrosis and a decrease in the degree of interstitial fibrosis in the corticomedullary junction. To determine whether this was due to changes in urine flow rate induced by enalapril, a group of animals was injected with BEA, and enalapril at the above dose was begun 1 wk later. After 1 mo, the enalapril-treated animals showed the same improvement in creatinine clearance (BEA 0.33 versus BEA + Enal 0.50 ml/min per 100 g body wt, \(P = 0.03\)) and suppression of albumin excretion. The area of papillary necrosis was reduced by 67%. In the BEA animals treated with enalapril, ED-1-positive cells, \(\alpha\)-smooth muscle actin, and transforming growth factor-\(\beta\) were decreased compared with BEA alone. It is concluded that in this model of papillary necrosis, enalapril protects renal function and decreases interstitial fibrosis mediated at least in part through an angiotensin II/bradykinin-independent mechanism. (J Am Soc Nephrol 9: 1052–1059, 1998)

Renal papillary necrosis is a finding frequently associated with analgesic abuse, diabetes mellitus, sickle cell disease, and urinary obstruction (1). In the rat, this lesion may be reliably induced by a single, 50-mg injection of 2-bromoethylamine (BEA). Arruda et al. (2) described in detail the functional characteristics of this lesion at 3 d and showed that it is associated with a profound defect in urinary concentration, a modest decrease in GFR, and impaired ability to conserve sodium and excrete a potassium load. Other investigators showed that acute BEA-induced papillary necrosis led to the development of progressive interstitial fibrosis and renal scarring (3,4). The mechanisms responsible for the progressive interstitial fibrosis in this model have not been investigated, and there are no long-term functional studies of this associated interstitial fibrosis.

Recent studies of interstitial fibrosis induced by ureteral obstruction (5), cyclosporin A (6), or immune complexes (7) have shown that inhibition of angiotensin II before or concurrent with the induction of a lesion can decrease the amount of fibrosis, as evidenced by a decrease in collagen deposition and interstitial cell infiltration. The purpose of this study was to determine the effect of the angiotensin-converting enzyme (ACE) inhibitor enalapril on the functional and morphologic outcome of BEA-induced papillary necrosis.

Materials and Methods

Experimental Design

Male Sprague Dawley rats (Harlan) weighing 250 g were used in all experiments. Animals were housed individually on a standard 12:12 light/dark cycle and had free access to standard rat chow (PMI no. 5012, PMI Feeds, Inc., St. Louis, MO) and drinking fluid. In the groups receiving enalapril, standard tap water was replaced with a solution initially containing 100 mg/L enalapril maleate (Sigma, St. Louis, MO). Intake was monitored daily, and the concentration of the enalapril solution was adjusted such that each animal received approximately 5 mg of drug per day. This was necessary because animals receiving BEA markedly increased their fluid consumption.

- Group 1 – Control: No treatment, injected intraperitoneally with 0.5 ml of saline (\(n = 7\)).
- Group 2 – ACE inhibition: Injected intraperitoneally with 0.5 ml of saline and drank enalapril for 4 wk (\(n = 6\)).
- Group 3 – Papillary necrosis: Injected intraperitoneally with 50 mg of BEA (\(n = 7\)).
- Group 4 – ACE inhibition pretreatment: Rats started on enalapril 1 wk before injection of BEA and enalapril continued for 4 wk (\(n = 10\)).
• Group 5 – ACE inhibition postinjection: Animals injected with BEA and enalapril started 1 wk later. Enalapril was continued for 4 wk (n = 5).

Balance studies were done weekly on all animals for the duration of the experiment. At the end of 1 mo, a final 24-h urine collection was done, followed by a 2-h collection for clearance studies. Animals were anesthetized with sodium pentobarbital (50 mg/kg), and a blood specimen was obtained from the inferior vena cava. The kidneys were perfused retrogradely with cold saline followed by 50 ml of Histo-Choice (Amresco, Solon, OH) and processed for routine histology. Animal experiments were conducted in accord with National Institutes of Health guidelines for the care and use of laboratory animals.

**Biochemical Studies**

Urine and plasma urea nitrogen and creatinine were measured by standard methods. GFR was assessed by creatinine clearance in conscious animals. To document that the changes in creatinine clearance truly reflected the changes in GFR, in a separate group of anesthetized animals we compared baseline creatinine clearance to inulin clearance. The ratio of the clearances was close to unity. The GFR was then changed by varying the arterial pressure. Both the inulin clearance and creatinine clearance were able to detect the change in GFR by the same magnitude (r² = 0.848, P = 0.009), although creatinine clearance overestimated it by approximately 10%. Urine albumin was measured by radial immunodiffusion on plates obtained from The Binding Site (Birmingham, United Kingdom).

**Immunohistochemistry**

Macrophages were identified with a mouse monoclonal anti-ED-1 antibody (Harlan Bioproducts, Indianapolis, IN). Positively stained cells were counted in 20 adjacent X40 fields at the corticomedullary junction. α-Smooth muscle actin (α-SMA) was localized by means of a mouse anti-actin, smooth muscle antibody (BioGenex, San Ramon, CA). Anti-Transforming growth factor-β1 (TGF-β1) was obtained from Promega (Madison, WI). Stained sections were evaluated by density measurements made using National Institutes of Health Image. Secondary antibodies were from Sigma and were visualized with Fast Red Naphthol, also from Sigma.

**Morphometric Analysis**

The extent of papillary necrosis was evaluated on periodic acid-Schiff-stained digitized coronal sections. These images included the tip and approximately three-fourths of the papilla. This area was divided by the total area of the papilla measured. Because of the focal nature of the lesion at 1 mo, interstitial fibrosis was evaluated at the corticomedullary junction, an area reported by others (8) to be the region of most severe damage at this time point. Contiguous fields at the corticomedullary junction were photographed at X4 and digitized. In a blind manner, the area of fibrosis, including the interstitial cell infiltrate and atrophic tubules, was measured and expressed as a percentage of the total area. The method was confirmed in a blind study on the same sections as evaluated by three pathologists who did not have prior knowledge of the condition of the animals.

**Table 1.** Urine volume and specific gravity 1 mo after BEA administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>U_vol (ml/h)</th>
<th>Sp.gv.</th>
<th>Rat Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7</td>
<td>0.50 ± 0.06</td>
<td>1.048 ± 0.003</td>
<td>340 ± 20</td>
</tr>
<tr>
<td>BEA</td>
<td>8</td>
<td>1.30 ± 0.23b</td>
<td>1.020 ± 0.002b</td>
<td>349 ± 9</td>
</tr>
<tr>
<td>Enal</td>
<td>6</td>
<td>0.80 ± 0.20</td>
<td>1.032 ± 0.002b</td>
<td>373 ± 6</td>
</tr>
<tr>
<td>Enal + BEA</td>
<td>10</td>
<td>1.43 ± 0.31b</td>
<td>1.026 ± 0.002b</td>
<td>371 ± 3</td>
</tr>
<tr>
<td>BEA + Enal</td>
<td>5</td>
<td>1.38 ± 0.17b</td>
<td>1.020 ± 0.004b</td>
<td>357 ± 15</td>
</tr>
</tbody>
</table>

* BEA, bromoethyamine; U_vol, urine volume; Sp.gv., specific gravity; Enal, enalapril.

b Significantly different from control at P = 0.02 or less.

**Figure 1.** Creatinine clearances at 1 mo. Enal + BEA: Enalapril for 1 wk before 2-bromoethylamine (BEA). BEA + Enal: Enalapril begun 1 wk after BEA. *P < 0.05 compared with control; **P < 0.05 compared with BEA alone.

**Figure 2.** Urine albumin-to-creatinine ratios. Control values, which ranged from 0 to 0.22, have been omitted for the sake of clarity. —■—, BEA; — - —, Enal + BEA; ----, BEA + Enal.

 lesion extend beyond this range. The cumulative area of periodic acid-Schiff-positive staining was measured and that number was divided by the total area of the papilla measured. Because of the focal nature of the lesion at 1 mo, interstitial fibrosis was evaluated at the corticomedullary junction, an area reported by others (8) to be the region of most severe damage at this time point. Contiguous fields at the corticomedullary junction were photographed at X4 and digitized. In a blind manner, the area of fibrosis, including the interstitial cell infiltrate and atrophic tubules, was measured and expressed as a percentage of the total area. The method was confirmed in a blind study on the same sections as evaluated by three pathologists who did not have prior knowledge of the condition of the animals.

**Statistical Analyses**

Except as indicated data are expressed as mean ± SD. Statistical analyses (ANOVA and regression) were performed using the program Minitab (State College, PA).
Results

Table 1 is a summary of the data in the different groups at 1 mo. In the BEA-treated animals, urine volume increased to more than twice that of the controls, and urine-specific gravity decreased strikingly. The ACE inhibitor enalapril tended to increase urine volume, although not significantly. The animals receiving enalapril before or after BEA had a significant increase in urine volume and a decrease in specific gravity compared with the controls, suggesting that enalapril did not completely prevent papillary necrosis. Indeed, the values obtained in the last two groups were not significantly different from those in animals receiving BEA alone. Body weight was not markedly different among the various groups.

Plasma creatinine concentration was significantly higher in the BEA animals compared with controls (BEA 0.84 ± 0.19 versus control 0.50 ± 0.13 mg/dl, P = 0.002). Ingestion of enalapril (Enal) before or after BEA was associated with a lower plasma creatinine compared with BEA-treated animals (Enal + BEA 0.76 ± 0.17; BEA + Enal 0.69 ± 0.11 mg/dl), although the difference did not achieve statistical significance.

Figure 1 shows the creatinine clearance corrected for 100 g body wt in the different groups studied at 1 mo. BEA admin-
administration decreased GFR by 50% (control 0.66 versus BEA 0.33 ml/min per 100 g body wt, \( P = 0.02 \)). Enalapril alone also tended to decrease GFR, but the difference did not reach statistical significance. Giving enalapril either before or after BEA led to a striking improvement in creatinine clearance compared with the animals receiving BEA alone (BEA 0.33 versus Enal + BEA 0.52, \( P = 0.01 \); BEA + Enal 0.50 ml/min/100 g, \( P = 0.03 \)). The creatinine clearances in these two groups were not significantly different from the control or enalapril-treated animals. Thus, enalapril treatment before or after BEA improved GFR significantly.

Figure 2 shows the albumin-to-creatinine ratio measured at weekly intervals after administration of BEA or enalapril + BEA. BEA administration led to a striking rise in the albumin-to-creatinine ratio at 7 d, diminishing after 14 d, but remaining higher than the controls at 1 mo (BEA 0.51 ± 0.40 versus control 0.19 ± 0.20, \( P < 0.05 \)). Enalapril before or after BEA blunted the early rise in albuminuria at 7 d, but clearly did not prevent this rise completely. Notably, albuminuria was undetectable by 3 wk in the BEA animals treated with enalapril. Albumin excretion for 24 h showed similar results (at 1 mo: Control 2.81, BEA 6.15, Enal + BEA 0, BEA + Enal 0.38 mg/24 h).

Figure 3 shows the papillary area in the four groups of animals at 1 mo. In the BEA-treated animals, 100% of the papilla was fibrotic (Figure 3D). In the animals receiving enalapril + BEA, the area of papillary necrosis was strikingly reduced (Figure 3, B and C). Figure 4 depicts the total area of the papilla measured, with the area of the papillary necrosis displayed in black. The percentage of the total area of papilla that was fibrotic was significantly lower in the animals treated with enalapril than those given BEA alone (BEA 100%, Enal + BEA 12%, BEA + Enal 33%). Thus, although enalapril treatment did not completely prevent BEA-induced papillary necrosis, it significantly decreased the magnitude of the lesion, as assessed by the area of fibrosis, GFR changes, and albuminuria. The changes in the creatinine clearance correlated inversely with the degree of papillary necrosis (\( r = 0.77, P < 0.001 \)).

In the light microscopic sections of animals receiving BEA alone, there was marked interstitial fibrosis and tubular atrophy.
junction and with sparing at the poles. By contrast, in enalapril-treated animals (Figure SB), there was a marked decrease in the deep nephron dropout, more evident at the cortical medullary interface. These cells were not limited to the corticomedullary junction but were also seen in the peritubular areas of the cortex. The ED-1 marker stained only approximately 40% of the mononuclear cells present. The unstained mononuclear cells are presumed to be lymphocytes. The number of ED-1-positive cells was reduced by enalapril, whether given before or after BEA (Figure 7B and Table 2).

We also performed immunostaining for α-SMA, a marker for interstitial fibrosis. In BEA-treated animals, there was positive staining of cells in the interstitium, not associated with vessels or the medullary rays. This staining was diminished or absent in the enalapril-treated animals (Figure 8, A and B).

The results of the TGF-β1 immunostaining are shown graphically in Figure 9. In the control animals, positive staining was seen mainly in the proximal tubules, whereas the glomeruli and collecting ducts were negative. One month after the administration of BEA, the area adjacent to the fibrosis showed an increase in TGF-β1 staining, whereas the fibrotic area itself was below control level (Figure 10A). The scanning densitometric measures included both areas. This may account for the apparently small increase in the BEA animals. Giving enalapril before or after BEA significantly reduced the staining of TGF-β1 (Figure 10B); however, the decrease was of a smaller magnitude if enalapril administration was delayed for 1 wk. Enalapril alone also significantly reduced staining.

### Discussion

In this study, we examined the functional and morphologic correlates of BEA-induced papillary necrosis. Although long-term studies of the histopathology in this model have been well described (3), the functional changes of this lesion at 1 mo have not been studied in detail. This study showed that the papillary necrosis induced by BEA is associated with marked interstitial fibrosis, a striking reduction in GFR, and the development of albuminuria. Urinary albumin increased markedly in the first week and then decreased, although remaining above control levels by threefold. The reason for the early rise in proteinuria was not clarified by the present study. Giving enalapril, either before or after BEA, largely prevented the decline in GFR. Albuminuria was less marked than when BEA was given alone, and at the end of 3 wk albumin could no longer be detected in the urine of the enalapril-treated animals. This is comparable to the observations of others on the renoprotective effects of ACE inhibitors (9–12).

Animals receiving only enalapril also showed a slight, although not statistically significant, decline in creatinine clearance at 1 mo. One cannot rule out the possibility that this decline was related to a decrease in BP in these otherwise normal animals. This decline is also seen in patients treated with ACE inhibitors (13,14). A recent study by Apperloo et al. (15) suggested that this initial fall reflected a lowering of the intraglomerular pressure. They concluded that this initial decline in GFR was an adequate predictor of the success of the renoprotective effect of ACE inhibition because it was positively correlated with a favorable outcome.

The role of angiotensin II has been well studied in obstructive nephropathy, in which the administration of enalapril before or concurrent with this lesion resulted in a decline in interstitial fibrosis and in the accompanying macrophage infiltration (16). Interestingly, in our study the administration of enalapril did not completely prevent the development of papillary necrosis, as shown by the results in Figures 3 and 4, and by the fact that the urine volume still was elevated and the specific gravity decreased to the same extent as in the animals treated only with BEA. The percentage of papillary necrosis, however, was strikingly reduced by enalapril, either pre- or

### Table 2. Number of ED-1-positive cells in the corticomedullary junction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>108 ± 32</td>
</tr>
<tr>
<td>BEA</td>
<td>5</td>
<td>2207 ± 676*</td>
</tr>
<tr>
<td>Enal + BEA</td>
<td>3</td>
<td>122 ± 51</td>
</tr>
<tr>
<td>Enal</td>
<td>6</td>
<td>960 ± 229b</td>
</tr>
<tr>
<td>BEA + Enal</td>
<td>5</td>
<td>1020 ± 156b</td>
</tr>
</tbody>
</table>

*a Significantly different from control at P = 0.005 or less.

Abbreviations as in Table 1.

*b Significantly different from BEA at P = 0.005 or less.

(Figure 5A). This was characterized by an infiltrate of mononuclear cells, increased matrix deposition, tubular dilation, and deep nephron dropout, more evident at the cortical medullary junction and with sparing at the poles. By contrast, in enalapril-treated animals (Figure 5B), there was a marked decrease in the mononuclear cell infiltrate and the interstitial fibrosis. The results of the morphometric study are shown in Figure 6. Enalapril administration, whether before or after BEA, significantly reduced the amount of interstitial fibrosis (BEA 26.6 ± 5.3, Enal + BEA 7.3 ± 6.4, BEA + Enal 6.0 ± 3.5, Enal alone 3.2 ± 2.3% of the total corticomedullary area).

Table 2 summarizes the number of ED-1-positive cells. As expected, the control animals had a minimal number of these cells (108 ± 32/mm²). In BEA animals, there was a striking increase in the number of ED-1-positive cells (Figure 7A). These cells were not limited to the corticomedullary junction but were also seen in the peritubular areas of the cortex. The ED-1 marker stained only approximately 40% of the mononuclear cells present. The unstained mononuclear cells are presumed to be lymphocytes. The number of ED-1-positive cells was reduced by enalapril, whether given before or after BEA (Figure 7B and Table 2).
Figure 7. ED-1 staining. (A) One month after BEA, there is an increase in the number of positive cells in the cortex. (B) If enalapril is given 1 wk before BEA, there is a significant decrease in the number of positive cells. Magnification, ×100.

Figure 8. Immunostaining of α-smooth muscle actin (α-SMA). (A) After BEA, α-SMA is found diffusely throughout the involved interstitium. (B) Enalapril administration is associated with a reduction in the amount of α-SMA. Here, staining is confined to a medullary ray. Sections have been counterstained with hematoxylin for clarity. Magnification, ×400.

post-BEA treatment. The mechanism by which enalapril exerts this effect is unclear. It could be speculated that because enalapril tended to increase the urine flow rate, that this was protective against concentrating BEA in the papilla, which might lead to the development of the necrosis. This, however, is not the sole explanation (or even the likely one), because the animals receiving enalapril 1 wk after BEA also had a similar reduction in papillary necrosis. This observation strongly suggests that the effect of enalapril to reduce the magnitude of papillary necrosis is neither mediated by a diuretic effect nor modulated through a direct hemodynamic effect of angiotensin II Bradykinin in the papilla.

This would be consistent with the work of others who have shown that although a cocktail of reserpine, hydralazine, and hydrochlorothiazide (17) or a calcium channel blocker (18) was as effective as ACE inhibition in reducing systolic BP,
Angiotensin has been shown to play an important role in the development of interstitial fibrosis, probably by stimulating TGF-β1 production (21,22). In this study, administration of BEA led to an increase in TGF-β1 in the normal tubules surrounding the regions of fibrosis and tubular atrophy, whereas the areas of most severe damage had expression below that of the controls. The overexpression of TGF-β1 is associated with increased matrix production and upregulation of protease inhibitors (23,24). The decrease in TGF-β1 in animals receiving enalapril is consistent with reports by others of the effect of ACE inhibition (5,25).

A possible role for bradykinin in this model cannot be ruled out. Bradykinin is generated by components of the vascular wall during ACE inhibition (26) and may stimulate nitric oxide production. Nitric oxide neutralizes superoxide anions, and it has been speculated that this may contribute to the cardioprotective effects of bradykinin in reperfusion-induced vascular injury (27). Morrissey et al. have proposed this as a mechanism for the renoprotective effect of ACE inhibition in the tubulo-interstitial fibrosis of obstructive nephropathy (28).

In summary, in addition to decreasing the magnitude of the papillary necrosis, enalapril pre- or post-BEA administration was associated with an improvement in GFR, a lesser degree of fibrosis, and a striking reduction in albuminuria. Taken together, these results strongly suggest a role for angiotensin II/bradykinin in the development of the morphologic changes and functional consequences of BEA-induced papillary necrosis. It is unknown whether other chemical mediators can exert the same protective effect.

**Acknowledgments**

The authors thank Shana Davis of the Morris Friedell Mentorship program and Leonid Slobodskoy for excellent technical assistance.
References