Quinapril Decreases Renal Endothelin-1 Expression and Synthesis in a Normotensive Model of Immune-Complex Nephritis

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Abstract. Angiotensin-converting enzyme (ACE) inhibitors diminish proteinuria and the progression to renal failure in several experimental models of renal injury. Endothelin-1 (ET-1) possesses potent biological actions on renal vessels and has been considered as a potential mediator of renal damage. Because angiotensin II (Ang II) induces ET-1 synthesis in endothelial and mesangial cells, we hypothesized that some of the beneficial effects of the ACE inhibitors could result from the blockade of ET-1 synthesis. In a normotensive model of immune-complex nephritis, in which there exists an increase in renal ACE activity, the effect of the ACE inhibitor quinapril on ET-1 protein and ETA receptor gene expression, as well as on ET-1 protein levels, was examined in this study. In relation to controls, nephritic rats showed an increase in preproET-1 and ETA receptor gene expression in renal cortex and medulla, coinciding with the maximal renal ACE activity. PreproET-1 mRNA (in situ hybridization) and ET-1 protein (immunohistochemistry) were localized in glomerular capillary walls, mesangial and glomerular epithelial cells, as well as in the brush border of some proximal tubules, and in small vessels. In nephritic rats, there was an increase in preproET-1 mRNA levels and ET-1 protein in all of these areas, without modification of their distribution. The administration of the ACE inhibitor quinapril decreased proteinuria and morphological lesions, preproET-1 gene transcription, and ET-1 protein levels, as well as the ETA receptor mRNA. The results from this study show that in a normotensive model of immune-complex nephritis, there was an overexpression of ET-1 in several structures of the kidney that was downregulated by quinapril administration. The beneficial effect of ACE inhibitors could be a result of the modulation of local production of Ang II and ET-1. (J Am Soc Nephrol 8: 756-768, 1997)

Angiotensin II (Ang II) and endothelin-1 (ET-1) are two vasoactive peptides that, besides their effects on renal hemodynamics, could participate in the pathogenesis of glomerulosclerosis through their actions on resident renal cells. Both peptides have similar biological actions, such as cell contraction, induction of early gene expression, cell proliferation, and extracellular matrix protein synthesis in various renal cells. Furthermore, profound connections seem to exist between the synthesis of both hormones. Thus, Ang II induces ET-1 synthesis in cultured endothelial cells and mesangial cells. On the contrary, in pulmonary artery endothelial cells, ET-1 stimulates the conversion of Ang I to Ang II. Moreover, an interrelation between its effects has been observed. In this sense, in mesangial cells, the proliferative response and matrix production induced by Ang II are mediated by ET-1 release.

Many studies have suggested that the renin-angiotensin system (RAS) is involved in the pathogenesis of renal damage. Early indications came from studies in which treatment with angiotensin-converting enzyme (ACE) inhibitors reduced proteinuria and sclerosis in experimental models of renal injury that were characterized by elevated systemic and glomerular capillary pressure. However, recent studies have emphasized the importance of inhibiting the local Ang II generation, even in situations of normal glomerular pressure. ET-1 plays an important role in the regulation of renal hemodynamics in physiologic and pathophysiologic conditions. In fact, recent studies have demonstrated an upregulation of renal ET-1 and its receptors in the models of renal mass ablation, diabetic nephropathy, and anti-Thy-1 glomerulonephritis, that was correlated with the progression of the disease. Further implication of ET-1 in those settings has been supported by the beneficial effect of endothelin receptor antagonists on the progression of renal disease.

Because systemic hypertension has a negative impact on kidney injury, the beneficial effects of both ACE inhibitors and endothelin receptor antagonists could not be differentiated from their hypotensive effects. For this reason, we turned our attention to a normotensive model of immune-complex nephritis in which serum ACE activity was normal, whereas renal ACE activity was elevated. This model allows dissociation of the systemic effects of vasoactive hormones from those...
Table 1. Effect of quinapril treatment on clinical and biochemical parameters in rats with immune-complex nephritis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control-Quinapril</th>
<th>Nephritis</th>
<th>Nephritis-Quinapril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (mg/day)</td>
<td>4.1 ± 0.5</td>
<td>3.1 ± 0.1</td>
<td>530 ± 53</td>
<td>82 ± 31</td>
</tr>
<tr>
<td>Mean pressure (mmHg)</td>
<td>121 ± 15</td>
<td>117 ± 14</td>
<td>119 ± 16</td>
<td>122 ± 10</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>235 ± 10</td>
<td>240 ± 20</td>
<td>218 ± 12</td>
<td>220 ± 15</td>
</tr>
<tr>
<td>Creatinine clearance (μl/min per 100 g)</td>
<td>307 ± 21</td>
<td>309 ± 20</td>
<td>236 ± 52</td>
<td>310 ± 33</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>63 ± 18</td>
<td>67 ± 21</td>
<td>335 ± 18</td>
<td>174 ± 27</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM of n = ten to 12 animals per group.

**P < 0.005, nephritic versus control rats.

***P < 0.005, quinapril-treated versus untreated nephritic rats.

exerted directly on renal cells. We have previously demonstrated that the administration of the ACE inhibitor quinapril decreased proteinuria, morphological lesions, and gene expression of transforming growth factor β (TGF-β) and extracellular matrix proteins (20). This suggests an important role of the local RAS in the pathogenesis of the glomerular and interstitial damage in this model. In this article, we have evaluated whether the beneficial effects of ACE inhibition on that immune-complex nephritis could result from its action on ET-1 synthesis. Therefore, we investigated the renal expression and synthesis of ET-1, as well as that of its receptors, and its modification by the ACE inhibitor quinapril.

Materials and Methods

Experimental Design

Studies were conducted in normotensive female Wistar rats with initial weights of 200 to 220 g. Immune-complex nephritis was induced according to a previously described protocol (21). In brief, rats received an initial subcutaneous injection of 5 mg of ovalbumin (OVA; Sigma Chemicals, St. Louis, MO) in complete Freund’s adjuvant (Difco, Detroit, MI), and 3 wk later, the same dose was given in incomplete Freund’s adjuvant (Difco). One week later, daily intraperitoneal administration of 10 mg ovalbumin was started. Proteinuria appeared around the ninth week. When proteinuria reached 20 to 50 mg/day, animals were randomly distributed into two groups: (1) the untreated group, composed of animals with spontaneous development of nephritis; and (2) the quinapril-treated group, composed of animals treated with the ACE inhibitor quinapril (a gift from Parke Davis as powdered hydrochloride) at a concentration of 100 mg/l, added to the drinking water and replaced every 48 h.

Three weeks after the onset of proteinuria, animals were weighed and euthanized, and blood was collected and kidneys removed. A parallel control group of animals of the same age, with or without treatment, was also studied.

Blood Pressure Measurement

Systolic arterial blood pressure was measured in conscious restrained rats by a tail-cuff sphygomanometer (NARCO Biosystems, Austin, TX). The blood pressure value for each rat was calculated as the average of three separate measurements at each session.

Kidney Tissue and Blood Processing

At the time of euthanization, the animals were fasted overnight and anesthetized with sodium pentobarbital (5 mg/100 g body wt). The kidneys were perfused in vivo via the abdominal aorta with 100 ml of normal saline at 4°C with a modified technique (22), with the additional step of opening the left femoral vein to permit the perfusate to drain. For ACE activity measurement, blood samples were collected and centrifuged (2000 g for 10 min). Serum was then obtained, and aliquots were preserved at −70°C. For ET determination, samples were collected in cold tubes containing EDTA (2 mg/ml) and aprotinin (500 U/ml), two protease inhibitors, and centrifuged (2500 rpm at 4°C for 15 min). Plasma was then collected and stored at −70°C until analysis. The kidneys were removed immediately and further processed for histological and in situ hybridization studies, ACE determination, and RNA extraction.

Biochemistry

Urine samples were collected periodically from rats kept in metabolic cages for 24 h with access to water but not to food. Proteinuria was measured by the sulfosalicylic acid method. Urine and serum creatinine levels were measured by an enzymatic method in a multichannel autoanalyzer. At the end of the study period, serum levels of creatinine, total protein, and cholesterol were determined according to standard methods. The creatinine clearance rate was calculated from three urine samples taken 72 h before the animal was euthanized.

Measurement of Endothelin in Plasma

ET was extracted from a 1-ml aliquot of plasma as previously described (23), using a Sep-Pak C18 disposable column (Waters, Milford, MA). The eluted fractions were evaporated in a N2 atmosphere, and the residues were stored at −20°C until the radioimmunoassay (RIA) was performed. ET-1 was measured in each sample by a commercial RIA (Peninsula Laboratories, Inc., Belmont, CA). The ET-1 antibody used in the RIA had a 7% crossreaction with ET-2 and ET-3 and a 17% crossreaction with the Big-ET (human). Crossreactivity with nonrelated peptides (atrial natriuretic peptide, brain natriuretic peptide, Ang II, vasopressin, and vasoactive intestinal peptide) was 0%.

Measurement of ACE Activity in Serum and Renal Tissue

Tissue for ACE activity examination was frozen in liquid nitrogen and stored at −80°C until its study. The ACE activity was determined as described previously (20). In brief, samples were homogenized, and the resulting supernatant was used for analysis of tissue ACE activity. ACE activity in tissue is expressed as relative units per milligram protein, as determined by the Lowry method (24) and as units per milliliter in serum.
Figure 1. Effect of quinapril treatment on glomerular damage in rats with immune-complex nephritis. (A) Photomicrograph showing a glomerulus from a control rat without morphological damage. (B) Glomerulus from a nephritic rat showing expansion of the mesangial area, cellular proliferation, focal sclerosis, and a crescent lesion. (C) Glomerulus from a quinapril-treated rat, showing only a slight increment in the mesangial matrix. (Hematoxylin-eosin stain; magnification, ×400.)
Figure 2. Semiquantitative analysis of glomerular and tubular damage in untreated (black bars) and quinapril-treated (white bars) nephritic rats. Results are expressed as mean ± SEM of eight animals per group. *P < 0.05 versus untreated rats. There was no difference between healthy control rats and quinapril-treated control rats (not shown).

RNA Extraction and Reverse Transcription

Total RNA was extracted from cortex and medulla by the acid guanidinium-phenol-chloroform method (25). The RNA was quantified by absorbance at 260 nm, and the absence of degradation was determined by electrophoresis of denatured RNA in 1% agarose-formaldehyde gel by ethidium bromide staining. One microgram of RNA from four rats of the same group was pooled and used as a single sample.

To obtain cDNA for the PCR, 1 μg RNA from each sample was transcribed in a final volume of 20 μl, which contained 5 mM MgCl2, RT buffer (10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton X-100), 1 mM deoxynucleotide mixture (dNTP), 20 U RNasin® (a ribonuclease inhibitor), 15 U of reverse transcriptase of the avian Mooney virus, and 50 ng random primer. The reaction mixture was incubated at 42°C for 45 min. At the end of the incubation, samples were heated at 95°C to eliminate transcriptase activity and to denaturalize the RNA-cDNA hybrids. Four microliters of cDNA templates was used for each PCR of preproET-1 and ETA receptor, and 2 μl was used for GAPDH.

PCR and Analysis of Its Products

PCR was performed with rat preproET-1, ETA, and ETB receptors and GAPDH specific oligonucleotide primers (Ramon Cornel, Madrid, Spain). PreproET-1 sense primer 5’-TGATCTTCTTCTTGCTGTTCGGG-3’ corresponded to nucleotides 17 through 40, and the antisense primer 5’-TCTTTTACGCCTTTCTGCGAGTAC-3’ corresponded to nucleotides 401 through 425 of the published sequence (26). ETA receptor sense primer 5’-GAAGTCTCCTGGGCGATCA-3’ corresponded to nucleotides 495 through 514, and the antisense primer 5’TCAAGAGACGCCCAAGACT-3’ corresponded to nucleotides 801 through 820, and the antisense primer 5’-ACGATGAGGACAATGAGATT-3’ corresponded to nucleotides 1345 through 1365 of the published sequence (28). We performed a RT-PCR of GAPDH as an internal standard. GAPDH sense primer 5’-AATGACTCACCACCCAA-3’ corresponded to nucleotides 439 through 458, and the antisense primer 5’-GTAAGCATATTCTGTGCATAA-3’ corresponded to nucleotides 934 through 954 of the published sequence (29). The cDNA amplification products were predicted to be 409, 216, 565, and 516 bp in length. Ten picomoles of sense and antisense primers, 0.5 μCi dCTP-α³²P (>3000 Ci/mmol; Amersham International, Buckinghamshire, UK), and 1.5 U Taq DNA polymerase were used per reaction. The reaction mixture (20 μl) was overlaid with mineral oil. The tubes were placed on a Thermal Cycler® (Perkin Elmer Cetus, Emeryville, CA), which was programmed as follows: incubation at 92°C for 30 s, then 30 cycles of the following sequential steps: (1) preproET-1: 92°C for 30 s, 59°C for 1 min, 72°C for 1 min; (2) ETA receptor: 92°C for 1 min, 60°C for 1 min, 72°C for 1 min; (3) ETB receptor: 92°C for 1 min, 57°C for 1 min, 72°C for 1 min; (4) GAPDH: 92°C for 30 s, 54°C for 1 min, 73°C for 1 min; and finally, incubation was done at 72°C for 7 min. The optimum number of amplification cycles used for quantitative RT-PCR was chosen based on pilot experiments that established the exponential range of each reaction. In all experiments, the presence of possible contaminants was checked by control reactions in which amplification was carried out in the absence of reverse transcriptase. PCR-amplified products were saved and kept at −20°C until analysis. The PCR products were size-fractionated with 1.5% agarose gel electrophoresis, and the DNA bands visualized with ethidium bromide staining. PCR products were blotted to Gene Screen (DuPont New England Nuclear, Boston, MA) in 0.4 N NaOH. Membranes were exposed to X-Omat AR films (Kodak, Rochester, NY) and intensifying screens at −70°C. The optical density of mRNA for PCR products on the autoradiograph was quantified using scanning densitometry (Molecular Dynamics, Sunnyvale, CA).

Renal Histopathological Studies

All histological studies were scored by two independent observers without their knowing to which group the animals belonged (double-blind study). The mean values were calculated for each rat. Renal tissue was fixed in buffered formalin and embedded in paraffin.

Light microscopy. Sections (3- to 4-μm thickness) were prepared and stained with hematoxylin-eosin, periodic acid-Schiff, and Masson trichrome stains. Mesangial expansion and glomerulosclerosis were defined with an arbitrary scale as previously described (20).

In situ hybridization. Digoxigenin-labeled single-strand RNA probes of preproET-1 were prepared using a nonradioactive RNA labeling kit (Boehringer Mannheim, Mannheim, Germany) according to manufacturer's instructions.
paraffin-embedded renal tissue was cut at a 4 μm thickness and floated onto APES (Sigma)-coated slides. The hybridization was performed with modifications of a previously described protocol (31). The tissue sections were heated at 65°C overnight and then fixed with 1.5% paraformaldehyde-1.5%glutaraldehyde. After they were dewaxed, tissues were incubated in 5 mM levamisole to inhibit endogenous phosphatase. Deproteinization was carried out in 0.2 N HCl, followed by digestion with proteinase K and two washings in 0.2% glycine for 5 min each time. After digestion, all sections were post-fixed as above, dehydrated through a graded ethanol series, and air-dried at room temperature. The slides were hybridized with 10ng/μl denatured digoxigenin-11-UTP-labeled riboprobes in hybridization buffer [2 × SSC, 1× Denhardt's solution, 0.1 M sodium phosphate (pH = 6.5), 10% dextran sulfate (in formamide)]. Sealed coverslips were placed over the tissue sections, and hybridization was allowed to occur overnight at 42°C in a moistened chamber. The coverslips were removed, and the slides were washed in 2 × SSC for 1 h at room temperature and 0.2 × SSC for 30 min at 37°C. The sections were incubated with an anti-digoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim) for 30 min at 37°C. Colorimetric detection of RNA-RNA hybrids was performed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate in the dark for 1 to 4 h. The color reaction was stopped with 10 mM Tris-HCl, 1 mM EDTA (pH = 8), and coverslips with 60% glycerol were then applied before the microscopic examination. The negative controls included: (1) hybridization with the sense probe, (2) RNase treatment before hybridization, and (3) omission of the RNA probe.

**Tissue localization of endothelin-like immunoreactivity.** Paraffin-embedded renal tissue was cut at 4 μm and mounted on poly-L-lysine-coated slides. Immunoperoxidase staining was performed by the avidin-biotin-peroxidase complex method (32). The slides were deparaffinized with graded concentrations of xylene and ethanol. The slides were quenched in methanol containing 3% H2O2 at 25°C for 30 min. The section were washed and incubated with trypsin (0.1% trypsin in 0.1% CaCl2, wt/vol) to activate antigenic sites. They were subsequently incubated in PBS with 5% normal swine serum in 5% nonfat milk for 1 h at 37°C to reduce nonspecific background staining and then incubated overnight at 4°C with rabbit polyclonal anti-END-1 antibody (Peninsula Laboratories) diluted 1:500 in PBS containing 1% normal swine serum and 5% nonfat milk. The control slides were treated with diluted normal rabbit serum. After being washed with PBS, the sections were incubated with biotinylated swine anti-rabbit immunoglobulin G (IgG; Dako A/S, Glostrup, Denmark) at a dilution of 1:200 and, after being washed, incubated with avidin-biotin-peroxidase complex (Dako A/S) diluted 1:100 in PBS for 30 min. The sites of peroxidase activity were visualized with 0.05% 3,3'-diaminobenzidine (Dako A/S) in 0.01% H2O2 for 10 min. The sections were counterstained with Mayer's hematoxylin (Sigma) and mounted in Pertex (Medite AG, Germany).

Approximately 15 glomeruli from each animal were examined, and the immunostaining was graded from 0 to 4+ by a semiquantitative score according to the following criteria: 0, no staining; 1+, minimal staining; 2+, moderate staining; and 3+, marked staining.

**Statistical Analyses**
Values reported are mean ± SEM. Comparisons between means of multiple groups were analyzed by one-way analysis of variance and t test. In all cases, differences were considered significant if the P value was less than 0.05.

**Results**

**Effect of ACE Inhibition on the Evolution of the Disease**
In this model of immune-complex nephritis, proteinuria appears 4 to 5 wk from the beginning of intraperitoneal administration of ovalbumin, and approximately 3 wk later, the
animals develop full-blown nephrotic syndrome and moderate renal failure (21,33).

**Effect on proteinuria and biochemical parameters.**
When proteinuria reached values between 20 to 50 mg/day, animals were randomly distributed into two groups: untreated (30 ± 12 mg/day) and quinapril-treated (31 ± 11 mg/day; P = not statistically significant). At the end of the study (21 days of treatment), in quinapril-treated rats, proteinuria was significantly reduced versus untreated rats (82 ± 31 versus 530 ± 53 mg/day; n = 12, P < 0.05) and the renal function was improved (Table 1). Biochemical parameters were also ameliorated in quinapril-treated rats (Table 1). Mean systolic blood pressure in all rats was in the normotensive range throughout the 3 wk of study, and the administration of quinapril did not significantly modify the systolic pressure (Table 1). There was no change in body weight along the period of study in all experimental groups (Table 1).

**Effect on morphological lesions.** In previous studies, we detailed the morphological aspects of glomerular lesions in this model (20,21). Untreated nephritic rats presented an increase in glomerular hypercellularity with segmental fibrinoid necrosis, mesangial matrix expansion, segmental and focal glomerulosclerosis, and mononuclear cell infiltration in the glomeruli and interstitium (Figures 1 and 2). After 3 wk of treatment with quinapril, a significant decrease in the glomerular (1.62 ± 0.2 versus 0.7 ± 0.1 untreated nephritis; n = 8, P < 0.05) and tubulointerstitial lesions was observed (1.1 ± 0.1 versus 0.05 ± 0.01 untreated nephritis; n = 8, P < 0.05) (Figures 1C and 2).

**Systemic ET-1 Levels and ACE Activity**
There was no increase in ET-1 plasma levels and serum ACE activity in nephritic rats, compared with control rats (Table 2). These results suggest that systemic RAS and ET-1 levels were not activated in nephritic rats. By contrast, renal ACE activity was increased in nephritic rats versus controls (Table 2).

**Renal Expression of preproET-1 and Endothelin Receptors mRNA**
When compared with that of control animals, total mRNA content of preproET-1 in cortex and in medulla was increased in untreated nephritic rats (threefold and twofold, respectively; n = 4 animals per group) (Figures 3 and 4). The administration of quinapril to animals with nephritis decreased preproET-1 mRNA expression, both in cortex and medulla (90% and 95% respectively, inhibition versus untreated nephritis; n = 4, P < 0.05) (Figures 3 and 4). This diminution was also observed in control animals that received quinapril (Figures 3 and 4).

In cortex and medulla, there was a slight increase in ETA receptor mRNA content in nephritic rats, compared with healthy control rats (1.4-fold and 1.5-fold, respectively; n = 4 animals per group) (Figures 3 and 4). The administration of quinapril induced an important diminution in ETA mRNA levels in both groups (90% and 85% respectively, inhibition versus untreated nephritis; n = 4, P < 0.05) (Figures 3 and 4). By contrast, ETB receptor mRNA levels were not modified in untreated nephritic rats, compared with healthy control rats (data not shown).

**In Situ Hybridization for preproET-1 mRNA**
The cellular distribution of preproET-1 mRNA in the kidneys of animals from different groups was investigated by *in situ* hybridization using digoxigenin-labeled riboprobes. In control experiments, hybridization with the sense riboprobe (Figure 5A), RNase treatment before hybridization or the omission of RNA probe did not label any renal structures. In
Figure 5. In situ hybridization studies. (A) Absence of labeling in renal rat tissue hybridized with a sense riboprobe for preproET-1. (B, C, D) Digoxigenin-labeled antisense riboprobe specific for preproET-1 hybridized under high-stringency conditions with kidney sections. (B) Cellular distribution of RNA in control rats. (C) Untreated nephritic rats. (D) Quinapril-treated nephritic rats. (Magnification, ×100.)
ACE Inhibition and Endothelin-1

Figure 6. Semiquantitative score of in situ hybridization studies in glomeruli (A) and tubules (B). Results are mean ± SEM of four animals per group. Staining was scored as (−), no staining; (1+), mild; (2+), moderate; and (3+), intense. The evaluation was done by two independent observers.

contrast, hybridization of kidney sections from normal rats with the antisense riboprobe yielded labeling in glomerular capillary walls, mesangial cells, and parietal and visceral glomerular epithelial cells, as well as in the brush border of some proximal tubular cells, and in small vessels (Figure 5B). A nearly identical labeling pattern of the renal structures was observed in rats with nephritis, although the intensity was stronger (Figure 5C and 6). The administration of quinapril decreased the mRNA expression of preproET-1 in all renal structures, both in control (not shown) and in nephritic animals (Figures 5D and 6).

**ET-1 Immunostaining**

To verify whether ET-1 gene expression in the kidney was followed by the synthesis of the corresponding protein, we studied the presence of ET-1 protein by immunoperoxidase, using a specific anti-ET-1 antibody.

In control rats, ET-1 immunoreactivity was localized in the glomeruli with a patchy distribution in the capillary wall (endothelial and epithelial cells) and in mesangial cells (Figure 7A). Focal immunostaining was also noted in the brush border of some proximal tubules and in Bowman’s capsule (Figure 8A). In untreated nephritic rats, the distribution of ET-1 was similar but had a greater intensity (Figures 7B and 8B). A certain staining was also seen in the endothelium of peritubular capillaries (Figure 8B). The administration of quinapril diminished the ET-1 staining in the different structures of the kidney (Figures 7C and 8C). No immunoperoxidase staining was observed in the samples from control and nephritic rats (treated and nontreated) when a non-immune serum was used (Figure 8D). The semiquantification of immunostaining for ET-1 in all groups studied is shown in Figure 9.

**Discussion**

In this work, we have demonstrated that the administration of the ACE inhibitor quinapril to normotensive rats with immune-complex nephritis downregulates renal preproET-1 mRNA and ET-1 protein levels, as well as the ETA receptor mRNA, coincidentally with a diminution in proteinuria, morphological lesions, and improvement in renal function. We explored the hypothesis that ET-1 could be one of the mediators of renal damage in situations characterized by increased local Ang II generation. We chose a normotensive model of glomerular immune injury with marked elevation of ACE activity in renal tissue and excellent clinical response to ACE inhibition, in spite of absence of elevated systemic blood pressure (20). This model is characterized by immune deposits in all glomerular areas, morphologically resembling human mesangiocapillary nephritis and active lupus nephritis (21,33). Initially, we studied the preproET-1 mRNA expression and its distribution in kidneys from rats with nephritis. In relation to control rats, nephritic rats presented an important increase in total renal preproET-1 gene expression that coincided with high renal ACE activity, marked nephrotic syndrome, and glomerular and tubulointerstitial lesions. This contrasts with the normal blood ET-1 levels and ACE activity along the evolution of the disease. By in situ hybridization, the increased preproET-1 gene was localized in glomerular capillary walls, mesangial cells, and parietal and visceral glomerular epithelial cells, as well as in the brush border of some proximal tubular cells, and in small vessels. By immunohistochemistry, an increase in ET-1 protein was also noted with the same distribution. Therefore, these results suggest that an increase in local generation of Ang II and ET-1 may participate in the pathogenesis of renal damage in this model of immune-complex disease.

The pathobiological significance of the enhanced renal expression and synthesis of ET-1 in renal injury remains elusive. Recently, it has been reported that kidney preproET-1 mRNA increases with the progression of renal damage in models so
Figure 7. Glomerular ET-1 immunostaining of control (A), untreated nephritic (B), and quinapril-treated (C) rats. In nephritic rats, ET-1 was localized in a focal distribution in the capillary wall (endothelial and epithelial cells) and in the mesangial area. A staining was also noted in the epithelial cells of Bowman's capsule. The same pattern, but less intense, was observed in control and quinapril-treated nephritic rats. Kidney sections were immunostained using specific ET-1 polyclonal antiserum. (Magnification, ×100.)
Figure 8. Tubular ET-1 immunostaining of control (A), untreated nephritic (B), and quinapril-treated (C) rats. In control rats, ET-1 was only detected in brush border of some tubules. In untreated nephritic rats, tubular dilation and atrophy, with some intraluminal casts, was noted. Therefore, an intense staining of ET-1 was observed in some tubules. Note that some capillary vessels were also stained. In quinapril-treated rats, a marked diminution in ET-1 staining was seen. (D) Untreated nephritic rats incubated with an unrelated IgG (negative control). (Magnification, ×50.)
Figure 9. Semiquantitative score of ET-1 protein in glomeruli (A) and tubules (B) in control and nephritic rats. The staining was scored as (−), no staining; (1+), mild; (2+), moderate; and (3+), intense. The evaluation was done by two independent observers. Results are expressed as mean ± SEM of six to eight animals per group. *p < 0.05 versus control rats; **p < 0.05 versus untreated nephritic rats.

In response to ACE inhibition, rats with nephritis presented a decrease in mRNA content of preproET-1 and ETA receptor in cortex and medulla in relation to untreated animals. By in situ hybridization, a downregulation of preproET-1 gene expression was also noted in all renal areas. The same was noted when the amount of ET-1 protein was studied by immunoperoxidase. Coincidentally, there was a significant decrease in proteinuria, glomerular and tubulointerstitial lesions, and a normalization of renal function. Those data are in concordance with the reduction in ET-1 gene expression in diabetic rat glomeruli after enalapril administration (36).

The beneficial effects of ACE inhibitors in reducing renal damage and ET-1 expression and synthesis could be a result of a locally decreased generation of Ang II. Although it may be argued that the modification in ET-1 could be a mere reflex of the intensity of tissue injury, we have recently seen that the administration of the dual ETA/ETB receptor antagonist bosentan improved proteinuria and renal lesions in this model (37). Also, a beneficial effect by ETA receptor antagonists was noted in experimental models of renal mass ablation and murine lupus nephritis (18,19). These data suggest that the blockade of ET-1 effects on renal cells could be responsible for the diminution of renal injury in those models.

There is some evidence that ET-1 might affect the RAS, although its role in renin release is controversial (38,39). In cultured pulmonary artery endothelial cells, the addition of ET-1 increased the conversion of Ang I to Ang II (8). Furthermore, ACE inhibitors can modulate the in vivo and in vitro effects of ET-1. Quinapril did attenuate the renal hemodynamic changes elicited by the intrarenal injection of ET-1 (40), and in cultured mesangial cells, ACE inhibitors decreased cell proliferation and matrix synthesis induced by ET-1 (10,41).

Both ETA and ETB receptors can be localized in the glomerulus by RT-PCR. The two receptors have different structures, functions, and distributions (42). However, the regulation and expression of both receptors in renal tissue during injury are scarcely known. In this work, we have observed an increase in the ETA receptor mRNA expression, both in cortex and medulla, during the progression of the disease, contrasting with the absence of changes in ETB gene expression. Several authors have reported that ET-1 produced by cells in culture can downregulate ET-1 receptors by an autocrine mechanism (43). However, an upregulation of ET receptor associated with increased preproET-1 mRNA levels has been observed in various diseases (34). Because ETA has been associated with cell proliferation and matrix protein synthesis (2,10), the increase in the ETA receptor mRNA expression in the kidney from animals with untreated nephritis, in the presence of unaltered ETB receptor levels, would further enhance these effects of ET-1 in renal cells. Interestingly, Ang II upregulates the expression of ETA receptors in cultured vascular smooth muscle cells (44).

Because ACE inhibition, besides reducing Ang II formation (45), also increases bradykinin concentration (46), with the subsequent generation of nitric oxide and prostaglandins (47), we may not discard the possibility that other mechanisms, independently of Ang II actions, could contribute to the de-
crease of the ET-1 expression. In this sense, recent data have shown that the ACE inhibitor captopril inhibits ET-1 secretion in endothelial cells by the accumulation of endogenous bradykinin and that this effect is mediated by its B₁ receptor (48). Further studies utilizing angiotensin or bradykinin receptor antagonists may be necessary to resolve this question.

On the whole, our results show that rats with immune-complex nephritis have an overexpression of ET-1 gene and ET-1 protein in several structures of the kidney, as well as the ETA receptor gene expression, that were downregulated by quinapril administration. The data presented here afford evidence that some of the in vivo Ang II effects could be mediated by ET-1. The beneficial effects of ACE inhibitors in progressive renal disease may be a result of both the diminution of glomerular hypertension and the modulation of Ang II and ET-1 effects on cell proliferation and matrix protein synthesis.

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References


