Gene expression in rats with renal disease treated with the angiotensin II receptor antagonist, eprosartan

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Wong, Victoria Y., Nicholas J. Laping, Lisa C. Contino, Barbara A. Olson, Eugene Grygielko, and David P. Brooks. Gene expression in rats with renal disease treated with the angiotensin II receptor antagonist, eprosartan. Physiol Genomics 4: 35–42, 2000.—The role of ANG II on renal and cardiac gene expression of matrix proteins was studied in rats with progressive renal disease. Induction of renal failure by five-sixths nephrectomy of Sprague-Dawley rats resulted in hypertension (163 ± 19 vs. control pressures of 108 ± 6 mmHg), proteinuria (83 ± 47 vs. 14 ± 2 mg/day), and increased renal expression of fibronectin, thrombospondin, collagens I and III, transforming growth factor-β (TGF-β), and plasminogen activator inhibitor-1 (PAI-1) mRNA. Treatment with the ANG II receptor antagonist, eprosartan (60 mg·kg⁻¹·day⁻¹), lowered blood pressure (95 ± 5 mmHg) and proteinuria (19 ± 8 mg/d) and abrogated the increased TGF-β, fibronectin, thrombospondin, collagens I and III, and PAI-1 mRNA expression. An increase in left ventricular weight was observed in five-sixths nephrectomized rats (0.13 ± 0.01 vs. 0.08 ± 0.01 g/100 g body wt), a response that was inhibited by eprosartan treatment (0.10 ± 0.01 g/100 g). Left ventricular expression of TGF-β and fibronectin was also increased in rats with renal disease; however, the small decreases in expression observed in eprosartan-treated rats did not reach statistical significance. These data suggest that eprosartan may be beneficial in progressive renal disease and that the mechanism of action includes inhibition of cytokine production in addition to antihypertensive activity.

transforming growth factor-β; renal disease; plasminogen activator inhibitor-1

ANGIOTENSIN II plays an important role in the long-term regulation of blood pressure; however, activation of the renin-angiotensin system and generation of the effector peptide ANG II has also been implicated in the progression of renal and cardiac diseases. There is growing evidence that the mechanisms by which the renin-angiotensin system contributes to disease progression involve organ and vascular remodeling in addition to the well-known vasoconstrictor activity. One of the main features of renal and cardiac remodeling is fibrosis, which is a complex process involving a number of different matrix proteins. In addition, there are cytokines involved in promoting matrix production, most notably transforming growth factor-β (TGF-β) (29), as well as genes involved in inhibiting the breakdown of matrix, for example, plasminogen activator inhibitor-1 (PAI-1). There is evidence that angiotensin can stimulate the production of both TGF-β (11, 18) and PAI-1 (14); however, it is unclear whether ANG IV or ANG II is more important in the PAI-1 response (20).

The present study was performed to determine whether a selective ANG II receptor antagonist, administered at a dose effective in attenuating renal disease-induced hypertension and proteinuria, would alter the expression of genes associated with renal and cardiac fibrosis.

MATERIALS AND METHODS

All procedures conformed to National Institutes of Health guidelines and were approved by the institutional Animal Care and Use Committee. Male Sprague-Dawley rats with initial body weights of ~250 g were used. Five-sixths nephrectomy was performed under pentobarbital anesthesia and sterile conditions. Via a midline incision, the right kidney was removed, and two of the three blood vessels supplying the left kidney were ligated, leaving approximately one-sixth of functioning kidney. Sham surgery was performed by making a midline incision but leaving both kidneys intact. Three weeks following surgery, rats were placed in the metabolism cages to collect 24-h urine samples. Following collection, urine was stored at ~20°C prior to assay. Urinary protein concentration was determined using the sulfosalicylic acid method (7), and 24-h urinary protein excretion was calculated. Systolic blood pressure was determined using tail plethysmography.

Starting 3 wk after 5/6 nephrectomy, eprosartan was administered for 4 wk intraperitoneally (~60 mg·kg⁻¹·day⁻¹) using two Alzet model 2 ML4 osmotic mini-pumps (Alza, Palo Alto, CA). Pumps containing vehicle were installed in control animals. Urinary protein excretion and systolic blood pressure were determined weekly for 3 wk following initiation of eprosartan treatment, at which time animals were killed and the kidneys and left ventricle were harvested. Tissues were dissected, weighed, frozen in liquid nitrogen, and stored at −80°C until RNA extraction. Total RNA was prepared from frozen tissues by guanidinium thiocyanate denaturation. RNA, 10 µg, was electrophoresed on 1% agarose gel after glyoxalation for 1 h at 55°C. RNA was transferred to Bright-
Staran treatment (Fig. 2). Renal TGF-

expression also appeared to be slightly lower in sham- 
operated rats treated with eprosartan (Figs. 2 and 3). Renal thrombospondin (Fig. 4), PAI-1 (Fig. 5), clusterin 
(Fig. 6), and collagens I and III (Fig. 7) mRNA levels were all significantly higher in rats following 5/6 nephrectomy. Eprosartan treatment resulted in significantly lower expression of all three.

Cardiac changes in gene expression were not as dramatic as the renal changes. There were significant increases of TGF-β and fibronectin in the left ventricle 
(Figs. 8 and 9); however, these responses were not

| Table 1. Body weight and left ventricular weight of 5/6 nephrectomized rats or control rats treated with eprosartan |
|-----------------|-----------------|-----------------|-----------------|
|                | Sham            | 5/6 Nephrectomized |
|                | Vehicle Eprosartan | Vehicle Eprosartan | Vehicle Eprosartan |
| Body wt, g     | 473 ± 12         | 439 ± 20         | 433 ± 9*         | 438 ± 14         |
| Left ventricular wt, g/100 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.13 ± 0.01a | 0.10 ± 0.01a |

Values are means ± SE; *P < 0.05 vs. sham. **P < 0.01 vs. sham.
altered by eprosartan to a degree that reached statistical significance (Figs. 8 and 9).

DISCUSSION

In the present study we have observed that progressive renal disease and hypertension induced by 5/6 nephrectomy was associated with increased cardiac and/or renal expression of TGF-β, fibronectin, thrombospondin, PAI-1, clusterin, and collagens I and III mRNA and that treatment with the ANG II receptor antagonist, eprosartan (5, 9), at a dose that attenuated the hypertension and proteinuria, resulted in a significant reduction in the renal expression of these genes.

It is becoming apparent that the mechanisms involved in the progression of renal disease may not be solely due to the increased glomerular hypertension that is secondary to ANG II-induced preferential efferent arteriolar vasoconstriction. This is an attractive hypothesis supported by a number of different studies (2, 8); however, others have suggested that increased matrix production rather than glomerular hypertension may be important (38). Thus ANG II, in addition
to its powerful vasoconstrictor effects, is able to stimulate the expression and/or production of a number of important profibrotic factors including TGF-β and PAI-1 (11, 18). TGF-β may be the single most important cytokine involved in enhanced matrix formation, because it is able to enhance both matrix protein synthesis and lead to inhibition of matrix breakdown (29). Our observation that blockade of the renin-angiotensin system with an AT$_1$ receptor antagonist can attenuate the enhanced TGF-β and matrix protein expression in renal disease is consistent with previous reports in a number of models of renal disease including partial nephrectomy (1, 17, 24, 36), immune-mediated renal injury (15, 37), mesangioproliferative glomerulonephritis (22, 39), hypertension-induced renal disease (25, 27, 35), unilateral ureteral obstruction (16), and cyclosporine nephrotoxicity (31). The possible role of ANG II in regulating PAI-1 expression in renal dis-

![Graph](http://physiolgenomics.physiology.org)

**Fig. 4.** Effect of eprosartan (60 mg·kg$^{-1}$·day$^{-1}$) on thrombospondin (TSN) expression following 5/6 nephrectomy or sham surgery. Equal loading of gels was determined using expression of ribosomal protein L32. *$P < 0.05$ vs. sham. †$P < 0.05$ vs. vehicle.

![Graph](http://physiolgenomics.physiology.org)

**Fig. 5.** Effect of eprosartan (60 mg·kg$^{-1}$·day$^{-1}$) on plasminogen activator inhibitor-1 (PAI-1) expression following 5/6 nephrectomy or sham surgery. Equal loading of gels was determined using expression of ribosomal protein L32. *$P < 0.05$ vs. sham. †$P < 0.05$ vs. vehicle.
ease, however, is less well characterized. PAI-1 is a serine protease inhibitor involved in the fast inhibition of tissue plasminogen activator and is produced by vascular endothelial cells and to a lesser extent by hepatocytes and platelets. In addition to being a prothrombotic factor, PAI-1, by virtue of its ability to inhibit matrix breakdown, is a potential mediator of fibrosis. The increased expression of PAI-1 observed in the present study is consistent with glomerular fibrosis observed in this model (4) as well as the presence of proteinuria. Indeed, it has been observed that circulating PAI-1 is increased in diabetic patients with albuminuria and that this is secondary to endothelial damage (13). The increased renal expression of PAI-1 following 5/6 nephrectomy suggests that it may play a role in glomerulosclerosis associated with progressive renal disease. Such a role is supported by our observation that the ANG II receptor antagonist, eprosartan, attenuated PAI-1 expression in addition to reducing proteinuria.

Inhibition of PAI-1 expression by an angiotensin type 1 (AT₁) receptor antagonist provides further evidence for a role of the renin-angiotensin system in the regulation of the plasminogen/plasmin system. It has previously been demonstrated that ANG IV stimulates PAI-1 expression in mesangial cells (19) and induces both PAI-1 expression and PAI-1 production in cultured endothelial cells (32). It has been proposed, however, that angiotensin-induced PAI-1 production and expression in vitro may be mediated by a receptor distinct from the AT₁ receptor, because the response cannot be inhibited by the AT₁-selective antagonist Dup-753 (losartan) (32). Furthermore, the hexapeptide ANG IV has been shown to induce PAI-1 expression in endothelial cells (20) and proximal tubule epithelial cells (10). This response may involve the putative AT₄ receptor, which has been identified using ANG IV binding activity in rat kidneys (14) and rabbit cardiac fibroblasts (33). Our data, however, do not support a major role for ANG IV in inducing cardiac or renal PAI-1 expression in vivo. Thus the increased PAI-1

Fig. 6. Effect of eprosartan (60 mg·kg⁻¹·day⁻¹) on clusterin expression following 5/6 nephrectomy or sham surgery. Equal loading of gels was determined using expression of ribosomal protein L32. *P < 0.05 vs. SHAM. †P < 0.05 vs. vehicle.

Fig. 7. Effect of eprosartan (60 mg·kg⁻¹·day⁻¹) on collagen I (top) and collagen III (bottom) following 5/6 nephrectomy or sham surgery as determined using quantitative polymerase chain reaction. *P < 0.05 vs. sham. †P < 0.05 vs. vehicle.
expression induced in the kidney by 5/6 nephrectomy was abolished by treatment with eprosartan. Eprosartan is a selective AT$_1$ receptor antagonist with no affinity for the putative AT$_4$ ANG IV receptor (R. M. Edwards, unpublished observations).

It is unclear from the present study whether the inhibition of PAI-1 expression by eprosartan is an effect involving blockade of ANG II on PAI-1 expression or an indirect effect involving TGF-β expression. In vitro, ANG II can result in both a rapid and prolonged increase in PAI-1 expression (19). The prolonged, but not the rapid, increase in PAI-1 expression can be blocked by a neutralizing antibody to TGF-β (19). It is possible, therefore, that the reduction in PAI-1 expression observed in the present study may be mediated, in part, by an indirect effect involving reduced TGF-β expression. Similarly, thrombospondin and TGF-β have been shown to enhance each other’s synthesis or activation (6, 12, 23, 28, 30), suggesting that the effect of ANG II blockade on thrombospondin expression is secondary to the effect on TGF-β. Nonetheless, this interaction highlights the feed-forward system that magnifies profibrotic mechanisms and how they can be interrupted using appropriate reagents.

In the present study, we observed a significant cardiac hypertrophy as evidenced by a 60% increase in leftventricular weight 4 wk following 5/6 nephrectomy. Our observation that eprosartan treatment prevented this supports the important role that the renin-angiotensin system plays in cardiac hypertrophy. Our data are consistent with a previous report evaluating an angiotensin receptor antagonist in rats with renal failure (24) and the observation that ANG II-selective antagonism can inhibit TGF-β gene expression and extracellular matrix in cardiac and vascular tissues of stroke-prone spontaneously hypertensive rats (21). It should be noted, however, that the changes in gene expression in the heart were less dramatic than the ones observed in the kidney and that the effects of eprosartan were modest; indeed, they did not reach statistical significance with the exception of PAI-1 mRNA, which was actually highest in the eprosartan-treated group. The reason for this is unclear. The reason for a greater response to eprosartan in the kidney may be due to higher local ANG II levels and thus a greater local effect. It is possible that a longer period of observation may have revealed a greater cardiac response; however, in the short term, the renin-angiotensin system appears to have a greater effect on renal remodeling than cardiac remodeling.

It is unclear from the present study what contribution the lowering of blood pressure had toward the reduction in expression of TGF-β, PAI-1, and the matrix proteins. If the effects of ANG II on these components are indeed receptor mediated, then it is unlikely that they can be separated from the blood pressure lowering effects. The observation that cardiac gene expression was reduced to a lesser extent than the renal expression suggests that mechanisms other than a reduction in blood pressure are indeed involved.
Consistent with this is the observation that carvedilol can reduce the renal damage in the spontaneously hypertensive stroke-prone rat without lowering blood pressure (3), indicating that a decrease in blood pressure is not necessary for renal protection. It is interesting that we have recently observed that carvedilol reduces renal TGF-β mRNA expression in spontaneously hypertensive stroke-prone rats treated with carvedilol (Wong et al., unpublished observations).

In summary, the data provided in this study provide further evidence that the beneficial effects of blocking the renin-angiotensin system involve effects on extracellular matrix components as well as cytokines that modulate extracellular matrix.

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REFERENCES


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