Mechanisms Mediating the Renal Profibrotic Actions of Vasoactive Peptides in Transgenic Mice

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Abstract. Transgenic mice are useful tools to investigate the mechanisms of the renal profibrotic actions of endothelin and angiotensin II. The overexpression of angiotensinogen and renin genes induces renal sclerosis independently of changes in systemic hemodynamics. The same results are observed when the endothelin-1 gene is overexpressed. Transgenic mice harboring the luciferase gene, under the control of the collagen I a2 chain promoter, and made hypertensive by induction of a nitric oxide (NO) deficiency have been studied. In this strain of mice, luciferase activity is an early index of renal and vascular fibrosis. Luciferase activity was increased in preglomerular arterioles and glomeruli when mice were treated with Nω-nitro-L-arginine methyl ester, an inhibitor of NO synthases. Bosentan (an endothelin receptor antagonist) was as efficient as losartan (an AT1 receptor antagonist) in preventing renal fibrosis, although it did not decrease BP. In short-term experiments, angiotensin II produced an increase in luciferase activity that was entirely prevented by losartan but also by bosentan. It can be concluded that, during chronic inhibition of NO, the collagen I gene is activated, which contributes to the development of nephroangiosclerosis and glomerulosclerosis. Angiotensin II plays a major role in this fibrogenic process, and its effect is at least partly independent of systemic hemodynamics and mediated by the profibrotic action of endothelin-1.

Renal vascular and glomerular injuries are frequent complications of human and experimental hypertension (1). Stimulation of extracellular matrix protein synthesis may occur as an adaptation to increased wall tension and/or to the action of vasoconstrictive peptides such as angiotensin II and endothelin-1.

Many in vitro studies support the hypothesis that angiotensin II regulates extracellular matrix formation independently of its vasoconstrictive action. For example, angiotensin II stimulates protein synthesis in cultured cardiac fibroblasts (2). In addition, endothelin-1 displays potent mitogenic properties and induces protein synthesis in cultured vascular smooth muscle cells and mesangial cells (3,4). Moreover, in various models of hypertension, in vivo administration of endothelin receptor antagonists limits end-organ damage but does not reduce high BP (5).

From these data, the question of whether renal fibrosis development and/or regression can occur independently of changes in systemic hemodynamics has been raised. If so, new therapeutic strategies could be proposed to prevent the progression of nephroangiosclerosis and glomerulosclerosis in human hypertension.

To gain new insights into the mechanisms mediating the renal profibrotic action of vasoactive peptides in vivo, several transgenic mouse models have been developed. In this review, we discuss the results of overexpression of genes for endothelin or renin-angiotensin components. We also examine data obtained using a transgenic mouse line that expresses luciferase under the control of the promoter of the mouse collagen type I gene (procoll I a2). This model allows a reliable and early index of renal fibrosis to be used in experimental models of hypertension.

Overexpression of the Renin-Angiotensin Components

The renin-angiotensin system has been the focus of the largest number of transgenic studies reported in the renal literature (6). Among numerous models, dual-transgenic mice expressing human angiotensinogen and renin genes under the control of the appropriate human promoters were developed to produce a new hypertensive model (7). These mice developed hypertension and renal fibrosis. Administration of lisinopril, an angiotensin-converting enzyme inhibitor, significantly decreased the renal glomerulosclerosis index without decreasing systolic BP. These results suggest that activation of the renal renin-angiotensin system induces renal sclerosis independently of systemic hypertension. Similar conclusions can be drawn from another study, which applied the unilateral ureteral obstruction model to a gene-targeting mouse line in which angiotensin II levels were varied from none to supraphysiologic levels through changes in the number of copies of the angiotensinogen gene (8). In these mice, renal collagen deposition depended on the number of angiotensinogen copies. Despite the fact that supraphysiologic levels of angiotensin II did not accelerate renal fibrosis development, the reduction of copies to zero or one limited renal collagen synthesis, compared with wild-type animals (with two copies), without decreasing sys-
tolic BP. These results clearly support the hypothesis that endogenous angiotensin II produced locally plays a role in the formation of renal fibrosis after ureteral obstruction, independently of alterations in systemic resistance.

Overexpression of the Endothelin Gene
Transgenic models have also been developed to study the influence of endothelin on BP and end-organ damage. When endothelin-1 gene overexpression occurred under the control of appropriate promoters, mice developed glomerulosclerosis and interstitial fibrosis, but the appearance of the renal lesions was not accompanied by changes in arterial pressure (9). Similarly, transgenic rat models that overexpressed the human endothelin-2 gene within the kidney exhibited glomerulosclerosis without increased BP (10). These transgenic lines are better models of pathophysiologic processes than are rats with overexpression of the human endothelin-1 gene under the control of a virus promoter in the liver (11). These data support a profibrotic action of endothelin peptides independent of their vasocostrictive properties. This is in accordance with the results of most pharmacologic studies testing the effects of endothelin receptor antagonists in hypertensive models (5), with the noteworthy exceptions of DOCA and Dahl’s models (12).

Reporter Gene under the Control of the Promoter of Procol I α2, an Index of Renal Fibrosis
Collagen type I expression is negligible in renal vascular structures under normal conditions (13). In contrast, this protein is highly expressed in nephroangiosclerosis and glomerulosclerosis (14,15). For this reason, the presence of collagen type I in preglomerular arterioles or glomeruli is an excellent index of vascular renal fibrosis. In our laboratory, we used a transgenic mouse line that expresses luciferase and β-galactosidase under the control of two sequences (−19.5 to −13.5 kb and −350 to + 54 pb) of the promoter of procol I α2. Previous data demonstrated that this construction contained a far-upstream enhancer regulating high levels of expression of the mouse procol I α2 gene (16). In preliminary experiments, we confirmed that the expression patterns of luciferase and β-galactosidase were correlated with the tissue distribution of collagen I under chronic conditions and with that of procol I α2 mRNA after acute stimulation of the collagen I gene.

This transgenic line offers specific advantages for studying renal vascular and glomerular fibrosis without interfering with the expression of the native collagen type I gene. In particular, measurements of luciferase activity allow mechanisms initiating in vivo fibrosis to be investigated under pathophysiologic conditions. This model has given us new insights into the involvement of angiotensin II and endothelin-1 in the renal damage induced by nitric oxide (NO) deficiencies (17,18).

To inhibit NO synthesis, mice were treated with Nω-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor (20 mg/kg per d), for 14 wk. This dose induced a gradual elevation of BP (Figure 1). In separate groups of mice, the mixed endothelin receptor antagonist bosentan or the angiotensin II AT1 receptor antagonist losartan was coadministered with L-NAME. Afferent arterioles and glomeruli were isolated from the transgenic mouse kidneys. Luciferase activity was measured in afferent arterioles, glomeruli, renal cortical slices, aortae, and hearts. Renal histologic analyses were performed using Masson trichrome solution for specific staining of extracellular matrix proteins. We observed that NO inhibition promoted procol I α2 expression in isolated afferent arterioles and glomeruli before the BP increase or the appearance of histologic alterations (Figure 1). In addition, procol I α2 gene activation was detected much earlier in the renal vasculature than in the aorta and the heart. Another important observation was that bosentan completely abrogated the L-NAME-induced activation of the collagen I gene in the renal vasculature during (8 wk) and after (14 wk) the establishment of hypertension, without affecting the increase in BP (17). Losartan was as efficient as bosentan in preventing renal damage induced by NO inhibition, although its effect on BP was absent at 8 wk and was only partial at 14 wk (18). Several conclusions can be drawn from these data. First, the development of renal fibrosis or its prevention is at least partly independent of changes in systemic hemodynamics. This observation does not exclude the possibility that the variations in the renal vascular tone are involved in the mechanisms controlling extracellular matrix synthesis. Second, constitutively produced NO negatively controls collagen I formation in the renal vasculature. Third, the beneficial effect of this autacoid is primarily explained by its inhibitory action on the profibrotic activity of angiotensin II and/or endothelin.

At this point in the discussion, three main questions may be asked, as follows. How is stimulation of the renin-angiotensin system explained when NO synthesis is inhibited? What are the respective roles of angiotensin II and endothelin in extracellular matrix production, inasmuch as losartan and bosentan are equally efficient in preventing renal fibrosis? Does transforming growth factor-β (TGF-β) play a role in the interaction between endothelin-1 and angiotensin II?

Interactions between NO and the Renin-Angiotensin System
The preventive effect of losartan on renal injury at the first step of L-NAME-induced nephropathy appears to be a paradox, because we (19) and others (20) demonstrated that NO suppression initially inhibited renin synthesis and renal angiotensin II production (21). One possibility is that the activity of the residual angiotensin II was potentiated by the absence of NO. Another explanation could be related to the dual regulatory action of NO on the renin-angiotensin system. In the presence of L-NAME, the low rate of renin production could be compensated for by an increased cellular effect of angiotensin II, through upregulation of AT1 receptors or through suppression of the NO-dependent inhibition of the intracellular angiotensin II pathway. These hypotheses are in accordance with the results of previous studies of vascular tissues (22,23).
Interaction between Angiotensin II and Endothelin-1

To answer the question regarding interactions between angiotensin II and endothelin, new experiments were performed. We incubated renal cortical slices and isolated aortae in the presence of angiotensin II. Angiotensin II produced an increase in luciferase activity, which was entirely prevented by the AT1 receptor antagonist losartan but also by bosentan. Moreover, urinary excretion of endothelin-1, which is considered an index of renal endothelin-1 production, was increased in L-NAME-treated mice, compared with control animals. This excess endothelin-1 production was less pronounced when mice were cotreated with losartan. Similar observations were made using other models of renal or vascular injury in which the renin-angiotensin system was activated. For example, it was reported that, in a transgenic model characterized by overexpression of renin and angiotensinogen, endothelin receptor blockade had no effect on BP but protected transgenic mice from vasculopathy and improved their survival rates (24). In rats, glomerulosclerosis observed in a model of angiotensin II-induced hyper-tension was reduced by endothelin receptor antagonists, whereas the effects of this treatment on BP were inconsistent (25,26).

These results support the involvement of endothelin-1 in the stimulatory effects of angiotensin II on renal collagen I synthesis and, more generally, in alterations of the vascular smooth muscle cell phenotype (Figure 2). They do not exclude the involvement of TGF-β, a potent profibrogenic agent, in renal sclerosis induced by angiotensin II (27). Using the same strain of mice, we investigated whether TGF-β played a role in the interaction between angiotensin II and endothelin-1. Acute in vivo administration of angiotensin II, endothelin-1, and TGF-β was performed in the presence or absence of their respective inhibitors, i.e., candesartan (an AT1 receptor antagonist), bosentan, and decorin (a scavenger of the active form of TGF-β). Our results suggested that endothelin-1 and TGF-β could stimulate the synthesis of collagen I independently of each other and that they operated in a synergistic way to mediate the angiotensin II-induced activation of procollagen 1α2 (28). Moreover, in aortic segments, angiotensin II activated the
collagen I gene through a mechanism involving TGF-β and the mitogen-activated protein/extracellular signal-regulated kinase MAP-ERK pathway, as demonstrated by the inhibitory effects of decorin and PD98059 (a blocker of the MAP-ERK cascade) on angiotensin II-induced luciferase activity (29). Finally, preliminary data indicated that TGF-β was overexpressed in rat renal cortex after 5 wk of treatment with l-NAME. This result is in accordance with the observations of Tomita et al. (30), who demonstrated, in the same model, that TGF-β expression was increased in cardiac fibroblasts and that this increase was prevented by AT1 receptor antagonism. It is likely that the renal sclerosis induced by l-NAME is explained not only by the activity of angiotensin II and endothelin-1 but also by the induction of TGF-β. Further experiments are needed to confirm this hypothesis.

Conclusion
The renal profibrotic actions of angiotensin II and endothelin-1 have been demonstrated in various experimental transgenic mouse models. These effects are independent of systemic hemodynamics. NO limits the fibrogenic process induced by these vasoactive peptides, whereas TGF-β likely acts in synergy with endothelin to favor the action of angiotensin II on the extracellular matrix. The observation that endothelin-1 mediates at least a part of the profibrotic effect of angiotensin II could have implications for the treatment of nephroangiosclerosis and glomerulosclerosis in human hypertension.

Acknowledgments
We thank Drs. Bou-Gharios, Rossert, and de Crombrugghe (Department of Molecular Genetics, University of Texas, Houston, TX), MSD-France, and Hoffman-Laroche for providing the transgenic mice, losartan, and bosentan, respectively. Experiments were financially supported by INSERM and Unité de Formation et de Recherche Saint-Antoine. Drs. Boffa and Fakhouri were research fellows of the Fondation pour la Recherche Médicale, and Dr. Tharaux was supported by the Groupe de Réflexion sur la Recherche Cardiovasculaire and the Assistance Publique-Hôpitaux de Paris.

References
5. Schiffrin EL: Role of endothelin-1 in hypertension. Hypertension 34: 876–881, 1999


