Angiotensin II and Renal Fibrosis

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Abstract—Angiotensin (Ang) II, the main peptide of the renin angiotensin system (RAS), is a renal growth factor, inducing hyperplasia/hypertrophy depending on the cell type. This vasoactive peptide activates mesangial and tubular cells and interstitial fibroblasts, increasing the expression and synthesis of extracellular matrix proteins. Some of these effects seem to be mediated by the release of other growth factors, such as TGF-β. In experimental models of kidney damage, renal RAS activation, cell proliferation, and upregulation of growth factors and matrix production were described. In some of these models, blockade of Ang II actions by ACE inhibitors and angiotensin type 1 (AT₁) antagonists prevents proteinuria, gene expression upregulation, and fibrosis, as well as inflammatory cell infiltration. Interestingly, Ang II could also be involved in the fibrotic process because of its behavior as a proinflammatory cytokine, participating in various steps of the inflammatory response: Ang II (1) activates mononuclear cells and (2) increases proinflammatory mediators (cytokines, chemokines, adhesion molecules, nuclear factor κB). Finally, Ang II also regulates matrix degradation. These data show that drugs controlling this complex vasoactive peptide are probably one of the best ways of avoiding fibrosis in progressive renal diseases. (Hypertension. 2001;38[part 2]:635-638.)

Key Words: angiotensin II ■ fibrosis ■ proteinuria ■ transforming growth factor

Treatment with blockers of angiotensin (Ang) II actions, such as ACE inhibitors or angiotensin type 1 (AT₁) antagonists, retards disease progression in humans and ameliorates proteinuria, inflammatory cell infiltration, and fibrosis in several models of kidney damage.1–3 In some of these settings, an activation of intrarenal renin-angiotensin system (RAS) has also been observed.1–3 There is now ample evidence to support the recommendation of ACE inhibition therapy as the standard of care for strategies aimed to preserve renal function in chronic renal failure (CRF).4,5 In addition, a better understanding of the nonhemodynamic actions of the RAS may lead to improved therapies for renal fibrosis.

The classical view of Ang II as a vasoactive agent that participates in local and systemic hemodynamic regulation has been recently enlarged to consider it as a true cytokine with an active role in renal pathology.6–8 As we review here, Ang II is a renal growth factor that modulates cell growth and extracellular matrix (ECM) synthesis and degradation.6–9 Glomerulosclerosis and interstitial fibrosis are key elements in end-stage renal failure. One common feature of progressive renal disease is the proliferation of resident renal cells, accumulation of ECM, and tissue retraction.10 Ang II seems to participate in these phenomena, although until present its role in tissue retraction has been poorly defined. As we discuss here, these processes seem to be mediated by the release of several factors, including transforming growth factor-β (TGF-β)11 and plasminogen activator inhibitor type 1 (PAI-1).12 Ang II may also participate in the fibrogenesis because of its implication in the inflammatory process through the synthesis of chemotactic factors, such as monocyte chemotactant protein-1 (MCP-1),8 and also because of its role in the induction of proteinuria13 (Figure 1).

Ang II and Mesangial Cell Growth and Matrix Synthesis Regulation

Mesangial cells are the main cells involved in the development of glomerulosclerosis. Ang II regulates mesangial cell growth, inducing proliferation or hypertrophy depending on the intracellular balance between growth factors,6 and increases the expression and synthesis of ECM proteins, such as fibronectin, laminin, and collagens.8 This increase in mesangial matrix is mainly mediated by TGF-β. In cultured mesangial cells, Ang II increases TGF-β mRNA expression and conversion to active form, and neutralizing antibodies to TGF-β remarkably reduce Ang II–induced ECM production.11 Systemic infusion of Ang II into normal rats increases glomerular TGF-β.11 In experimental models of renal damage upregulation of renal TGF-β expression associated with increased ECM, gene expression and matrix deposition have been described.2,3,11 ACE inhibitors and AT₁ antagonists reduced these abnormalities.2,3,11 Interestingly, systemic administration of TGF-β2 caused an elevation of tissue levels of Ang II, hypoxia, and renal fibrosis,14 showing the existence of a close interrelation between Ang II and the TGFβ system.

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Ang II and Tubulointerstitial Fibrosis

It has been largely demonstrated that the severity of tubulointerstitial changes correlated better with renal function compared with those of glomerulosclerosis. Interstitial nephritis is likely to evolve in stages involving inflammation, proliferation of interstitial fibroblasts, and excessive deposition of interstitial ECM, leading to fibrosis. Ang II may promote the phenotypic change of fibroblasts to myofibroblasts (α-smooth muscle actin-positive cells). These activated fibroblasts may proliferate and invade the periglomerular and peritubular spaces, contributing to matrix deposition in the tubulointerstitial area. Indeed, rats chronically infused with Ang II develop tubulointerstitial injury with tubular atrophy and dilation, cast formation, interstitial monocyte infiltration, and interstitial fibrosis with type IV collagen deposition. Cultured renal interstitial fibroblasts express AT1 receptors, and after Ang II stimulation, there is an increase in cell proliferation and expression and synthesis of ECM proteins, such as fibronectin, by a TGF-β-mediated mechanism. Platelet-derived growth factor-BB (PDGF-BB) and TGF-β1,8 were shown to induce transformation of fibroblasts into myofibroblasts. Several investigators, including ourselves, have demonstrated that the presence of interstitial myofibroblasts correlates with the extent of tubulointerstitial scarring and functional outcome in clinical glomerulonephritis. Renal tubular cells also play a central role in the pathogenesis of interstitial fibrosis. Tubular cells might be stimulated by the spillover of peptides such as Ang II, growth factors, and cytokines from the injured glomeruli. In cultured tubular cells, Ang II causes hypertrophy and also increases the synthesis of ECM via TGF-β.7 These cells also respond to proteinuria, producing many proinflammatory and profibrotic mediators, as well as ECM. However, the mechanism of this process is currently a subject of investigation. Rats with intense proteinuria presented elevated ACE and, therefore, increased local Ang II generation, mainly located in the proximal renal tubules, suggesting that proteinuria may promote tissue RAS activation that could be involved in the tubulointerstitial lesions of kidney diseases associated with persistent proteinuria.

Novel Mediators of Ang II–Induced Fibrosis

Emerging evidence suggests that connective tissue growth factor (CTGF), a novel profibrogenic cytokine, may be an important downstream mediator of TGF-β profibrotic activities. CTGF is generated in vitro in multiple cell types, including mesangial and tubular epithelial cells, by a variety of stimuli, such as high glucose. This novel growth factor is strongly upregulated by TGF-β, increases matrix production, is coexpressed in α-smooth muscle actin cells in the tubulointerstitial area, and appears to play a role in the development and progression of glomerulosclerosis and tubulointerstitial fibrosis. We have recently observed that in cultured renal cells, Ang II increases CTGF mRNA expression and synthesis. In addition, in nephritic rats, ACE inhibition diminished renal CTGF expression correlated with diminution of TGFβ and fibrosis (M. Ruiz-Ortega and J. Egido, unpublished data, 2001), showing that CTGF could be a mediator of the matrix accumulation caused by Ang II.

Ang II, Inflammation, and Fibrosis

Mononuclear cell infiltration of glomeruli and interstitium occurs in most progressive renal diseases and plays a crucial role in the outcome to irreversible structural changes. Several data suggest that Ang II could be involved in the inflammatory process during renal injury (Figure 1). Ang II activates inflammatory cells, by direct chemotaxis and production of proinflammatory mediators, including MCP-1 and TGF-β8. Pharmacological RAS blockade reduced of inflammatory cell infiltration in several models of renal injury. Pharmacologically Ang II infusion into normal rats caused mononuclear cell infiltration in glomeruli and interstitium. Ang II–infused rats presented tubular overexpression of osteopontin (OPN), a macrophage chemotactic and adhesion molecule, and glomerular overexpression of the chemokine RANTES, mainly located in endothelial cells. However, among chemokines, MCP-1 has emerged as a key mediator of monocyte infiltration. In cultured mesangial and mononuclear cells, Ang II is a potent activator of MCP-1 (mRNA and protein), to an extent comparable to that of other cytokines. Conditioned media from Ang II–treated mesangial cells possess chemotactic activity for monocytes that is, at least in part, inhibited by a neutralizing antibody against MCP-1. In experimental models of kidney damage, upregulation of renal MCP-1 coincidentally with mononuclear cell infiltration was noted. Both effects were markedly reduced by the treatment with ACE inhibitors. These data show that Ang II could contribute to the ongoing inflammation, facilitating the migration of mononuclear cells to the interstitium and glomeruli where they mature to macrophages and, ultimately, participate in the fibrotic process. These inflammatory cells would, in turn, activate renal cells through the release of a wide range of growth factors, including Ang II,
and therefore contribute to the perpetuation of kidney damage. Moreover, ECM production and/or deposition could also be influenced by the presence of the MCP-1, either directly as a fibrogenic factor or indirectly through TGF-β, and thus contributing to the development of fibrosis.31

**Ang II and Matrix Degradation**

The final fibrogenic phase is when ECM begins to accumulate, not only by upregulation of its synthesis but also by downregulation of its degradation. The impaired matrix turnover is caused by the renal production of protease inhibitors such as tissue inhibitors of metalloproteinases and PAI-1, which inactivate the renal proteases that normally regulate matrix turnover, leading to excessive matrix accumulation.12 The RAS is linked to the induction of PAI-1, possibly via AT₁ receptors,12 therefore facilitating both thrombosis and fibrosis.

**Ang II Receptors and Signal Transduction Pathways Involved in Renal Fibrosis**

Ang II acts through 2 specific receptors, AT₁ and angiotensin type 2 (AT₂). AT₁ regulates blood pressure, cell proliferation, and the production of cytokines and extracellular matrix proteins.32 AT₂ also regulates blood pressure control, renal natriuresis, cell growth inhibition, and renal inflammatory cell infiltration.32 Ang II, via AT₁, activates various nuclear transcription factors, including the activator protein-1 (AP-1), STAT family of transcription factors, and cAMP-response element–binding protein (CREB).8 Ang II also increases calcium release and activates protein kinase C (PKC), protein tyrosine kinases (PTK), mitogen-activating protein kinases (MAPK), extracellular signal-regulated kinase (ERK), c-Jun amino terminal kinase (JNK), p38 MAP kinase (p38 MAPK), and, as mentioned before, their downstream effector AP-1.32,33 MAPK are involved in proliferation, differentiation, fibrosis, and inflammation. In this sense, in Ang II–infused rats we have observed an elevation of renal AP-1 activity related to tubular damage that was diminished by the AT₁ antagonist losartan,25 and Ang II–induced type I collagen overexpression was mediated by AT₁ via MAP/ERK and TGFβ signaling pathways,34 suggesting that Ang II may regulate the fibrotic process via AT₁/AP-1 pathway. Different intracellular mechanisms regulate the AT₂-mediated cellular responses. AT₂ is linked to inhibition of MAPK, activation of protein-phosphotyrosine phosphatase (PPase), and ceramide production.32 We have observed that AT₁ and AT₂ share a common molecular pathway: the activation of nuclear factor κB (NF-κB).33 Both receptors share some signaling pathways such as redox-sensitive signals; however, PTK and MAPK only participate in AT₁/NF-κB responses.33 NF-κB regulates genes mediating cell growth control, inducing cell survival signals and protecting from apoptosis, but under certain conditions may also induce apoptosis.36 In vitro experiments suggest that Ang II may cause growth via AT₁ and apoptosis via AT₂.37 Among the intracellular mechanisms elicited by AT₁, ceramide production seems to be involved in apoptosis and NF-κB activation.32,35 Although many studies have been done, future work is necessary to completely understand the relation between AT/NF-κB/cell growth regulation. In vitro studies showed that in tubular cells, Ang II activated NF-κB only via AT₁, whereas in mononuclear and vascular smooth muscle cells both AT₁ and AT₂ were involved.25,35 In vivo, Ang II infusion increased renal NF-κB activity that was partially diminished by both AT₁ and AT₂ antagonists.25 Interestingly, both antagonists decreased NF-κB–positive staining in the glomeruli. Losartan markedly diminished NF-κB in tubular cells, whereas the AT₂ antagonist PD 123,319 did so mainly in inflammatory cells.25 In this sense, only AT₂ antagonists diminished Ang II–induced renal inflammatory cell infiltration25,27 and the induction of the chemokine RANTES in glomerular endothelial cells,27 showing a possible gene target for the AT₁/NF-κB pathway. Other important candidates of the AT₁/NF-κB pathway are inducible NO synthase and cyclooxygenase-2 (COX-2), which in inflammatory diseases are involved in NO/cGMP, and prostaglandin and thromboxane production, respectively.8 In vascular smooth muscle cells, the implication of AT₁/NF-κB pathway was confirmed using an AT₁ agonist and AT₁ knockout mice.35,38 In contrast, losartan markedly inhibited NF-κB-mediated transcription and gene expression, such as MCP-1, vascular cell adhesion molecule (VCAM), and interleukin-6, showing that proinflammatory genes are regulated by AT₁.8

All these data strongly suggest that activation of transcription factors such as NF-κB and AP-1 is involved in the pathogenesis of renal disease and document the complex mechanisms through which Ang II may cause induced tissue injury (Figure 2). In addition, the data also suggest that the beneficial effects in proteinuria, cell growth, fibrosis, and inflammation of RAS blockers (ACE inhibitors and AT₁ antagonists) may be owing, at least in part, to the decreased activation of these transcription factors. On the whole, we
have presented information showing that Ang II, by multiple mechanisms, is implicated in the process of renal fibrosis, and drugs controlling this complex vasoactive peptide are probably one of the best ways of avoiding fibrosis in progressive renal diseases.

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