Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases

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Abstract

Angiotensin II (AngII) participates in the pathogenesis of renal diseases, through the regulation of two key processes inflammation and fibrosis. AT1 and AT2 are the main receptors of AngII. AT1 mediates most of the actions of AngII. This receptor regulates...
the expression of profibrotic factors, such as connective tissue growth factor (CTGF). The Smad signalling pathway and the Rho/Rho kinase system are two novel mechanisms involved in AngII-induced matrix regulation recently described. The role of AT₂ receptors in renal pathophysiological processes is not fully elucidated. Experimental data suggest that AT₂ receptors through activation of nuclear factor-κB participate in renal inflammatory cell recruitment. Studies in animal models of kidney injury have shown that the combined blockade of both AT₁ and AT₂ receptors, as well as the inhibition of the NF-κB pathway are necessary to stop the inflammatory process fully. On the whole, these data highlight the complex signalling systems activated by AngII and suggest novel potential targets to block fibrosis and inflammation in renal diseases.

**Keywords:** angiotensin II; fibrosis; inflammation; proinflammatory cytokines; growth factors

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**Introduction**

Angiotensin II (AngII), the main peptide of the renin-angiotensin system (RAS), is involved in the pathogenesis of renal diseases [1,2]. This peptide acts through its binding to two specific receptors, AT₁ and AT₂ [2]. AT₁ is responsible for most of the pathophysiological actions of AngII. By promoting proliferation, inflammation and fibrosis, AngII contributes to chronic diseases, such as hypertension, atherosclerosis, cardiac hypertrophy and renal injury. The role of the AT₂ receptor is not completely defined.

AT₂ is involved in cell growth inhibition and inflammatory cell recruitment in the kidney [2–4]. We will review here the information regarding the novel mechanisms involved in the fibrotic and inflammatory response caused by AngII.

**AngII regulates fibrosis via the AT₁ receptor: role of CTGF and the Smad signalling pathway**

AngII via AT₁ regulates extracellular matrix (ECM) accumulation mediated by the endogenous production of profibrotic growth factors, such as transforming growth factor-β (TGF-β). Angiotensin-converting enzyme (ACE) inhibitors and AT₁ antagonists decrease tissue expression of TGF-β and fibrosis; furthermore, blockade of TGF-β diminishes AngII-induced ECM production [2,3]. Although TGF-β is one of the main regulators of fibrosis, therapeutic strategies blocking TGF-β actions have not afforded the expected beneficial effects probably because of its anti-inflammatory properties [5]. This is one of the reasons why novel antifibrotic targets are under active investigation. Connective tissue growth factor (CTGF) is a novel profibrotic factor that is upregulated in different human kidney diseases and contributes to renal fibrosis and tubulointerstitial transdifferentiation [6]. In models of renal injury, ACE inhibitors and AT₁ antagonists diminished CTGF upregulation and fibrosis [7,8]. We have also demonstrated that the blockade of CTGF, by an antisense CTGF oligonucleotide, diminished AngII-induced fibrosis, shown by diminution of fibronectin production [7,9]. These results suggest that CTGF could be a novel antifibrotic target (Figure 1).

![Fig. 1. Potential novel therapeutic strategies to block AngII-induced fibrosis. AngII via AT₁ activates the Smad signalling system and the Rho/Rho kinase pathway that upregulates CTGF production and fibrosis.](image-url)
The Smad proteins are essential components of the intracellular signalling pathways, acting as transcription factors of TGF-β-mediated responses, including fibrosis [10]. We have recently shown that AngII via AT_1 activates the Smad signalling system, independently of TGF-β [11]. In vascular smooth muscle cells (VSMCs), AngII caused a rapid and direct activation of Smad2 phosphorylation, nuclear translocation of phosphorylated Smad2 and Smad4, increased DNA-binding activity and Smad-dependent gene transcription. In AngII-infused rats, aortic Smad overexpression was associated with CTGF induction and occurred before ECM accumulation [11]. Smad7 may function as a general negative regulator of TGF-β receptor signalling [12]. Transient transfection with Smad7, which interferes with receptor-mediated activation of Smad2 and Smad3, diminished CTGF, fibronectin and type 1 procollagen upregulation caused by AngII [11]. Moreover, Smad7 overexpression blocks TGF-β-induced ECM production and renal fibrosis [13–15]. AT_1 blockade diminishes Smad pathway activation and fibrosis in the model of renal injury caused by unilateral ureteral obstruction (UUO), in myocardial infarction in rats and in the aorta of AngII-infused rats [11,16,17]. These data indicate that Smad signalling could be a common mechanism of AngII-mediated fibrosis in cardiovascular and renal diseases and that the blockade of Smad activation could be another important anti-fibrotic target (Figure 1).

**The small G protein Rho and AngII responses**

The AT_1 are G-coupled receptors and activate small G proteins, including RhoA and the Rho kinase system [18]. The Rho/Rho kinase signalling pathway participates in the development of fibrotic lesions in several tissues including the kidney. In different experimental models, such as hypertensive glomerulosclerosis, UUO, nephropathies and spontaneously hypertensive rats and L-NAME-treated rats, administration of Rho kinase inhibitors diminished glomerular and tubulointerstitial injury, inflammation and fibrosis, and downregulated smooth muscle α-actin gene overexpression, as well as the TGF-β and ECM proteins [19–24]. In rats infused with AngII, we have shown that the Rho kinase inhibitor Y-27632 diminished tubular damage, the number of inflammatory cells, and renal overexpression of CTGF and proinflammatory parameters [25]. All these data suggest that Rho kinase inhibitors could be novel targets for renal therapy (Figure 1).

**AngII and the inflammatory response: role of AngII receptors and the NF-κB pathway**

AngII contributes to the recruitment of infiltrating cells into the kidney; AngII causes the adhesion of circulating cells to endothelial and mesangial cells, and the migration of inflammatory cells into the kidney. This process is mediated by upregulation of adhesion molecules, cytokines and chemokines [1].

ACE inhibitors have been shown to diminish inflammatory cell infiltration and inflammatory markers in many animal models of renal injury [1]. AngII via AT_1 receptors upregulates many proinflammatory genes, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1), through the activation of several intracellular signalling systems, including the nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK) cascade, Rho proteins and redox pathways [1]. Some experimental data suggest that AT_2 receptors are involved in the inflammatory cell recruitment in the kidney. Only AT_2, but not AT_1, antagonists diminished the number of inflammatory cells in different animal models, including systemic infusion of AngII and UUO [3,4,8,26,27]. We have recently demonstrated that combined treatment with AT_1 and AT_2 antagonists blocked the inflammatory response, and lowered the number of infiltrating cells and the overexpression of proinflammatory genes to control levels in those models [8,27]. In the UUO model, AT_2 blockade diminished tumour necrosis factor-α (TNF-α) and RANTES overexpression, and the simultaneous blockade of both receptors abolished MCP-1 gene upregulation [8]. NF-κB activation has been described in kidney diseases [1]. *In vivo*, AngII activates the renal NF-κB pathway that was partially diminished by AT_1 or AT_2 antagonists alone, and was abolished by combination of both receptor antagonists or ACE inhibition [4,8,27]. In mesangial cells, NF-κB activation was mediated by both AT_1 and AT_2 receptors [4]; in tubuloepithelial cells, this was mainly by AT_1 [4], while in endothelial cells it was via AT_2 [28]. In the UUO model, we have found that blockade of renal NF-κB activation by treatment with two different NF-κB inhibitors, pyrrolidine dithiocarbamate (PDTC) and parthenolide, diminished the inflammatory cell infiltration and downregulated gene expression of several proinflammatory factors [8]. In spontaneously hypertensive rats, NF-κB inhibition attenuated renal interstitial inflammation and hypertension [29]. These data suggest that in some experimental renal diseases, the blockade of AngII generation by an ACE inhibitor or by combined blockade of both AT_1 and AT_2 receptors, as well as by the inhibition of the NF-κB pathway, is necessary to stop the inflammatory process fully (Figure 2).

In human kidney diseases, the activated renal renin–angiotensin system has been described. In diabetic nephropathy, elevated AngII generation did correlate with the presence of inflammatory cell infiltration, the activation of NF-κB and proinflammatory gene overexpression [30]. These observations emphasize the importance of treatments that block the AngII-induced inflammatory process in human renal diseases.
Drugs that modulate the renin–angiotensin system, such as ACE inhibitors and AT1 antagonists, have demonstrated protective renal effects and can ameliorate fibrosis. Current strategies in clinical practice combine treatments with ACE inhibitors and AT1 blockers, due to their potential additive beneficial effects [31]. However, these treatments did not cause regression of renal damage, suggesting that novel approaches are needed. The data presented here highlight potential interesting candidates for antifibrotic treatments, including CTGF, the Smad signalling system and the Rho/Rho kinase pathway. Future studies are necessary to evaluate their potential beneficial effects fully in kidney diseases.

The Ang receptor subtype, AT1 or AT2, involved in the inflammatory response in the kidney is not completely elucidated. Our results show that the blockade of both AT1 and AT2 receptors is necessary to stop the inflammatory process completely, at least in experimental models. The inhibition of the NF-κB pathway also prevents inflammation and experimental renal damage. All these experimental studies provide a rationale to investigate further the involvement of the AT2/NF-κB pathway in the inflammatory response in kidney diseases. These results could have potential clinical consequences in the treatment of severe human nephritis.

**References**

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Hypertensive myocardial fibrosis

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A variety of cardiac structural and functional changes, such as increased left ventricular mass (LVM), left atrial and aortic root enlargement, LV dysfunction, impairment of coronary reserve and prolonged ventricular repolarization, have been described in patients with long-standing arterial hypertension [1,2]. However, subtle modifications in LV structure and geometry may occur also in the early phases of the natural history of essential hypertension [3]. Among these manifestations of target organ damage, most attention has been devoted to LV hypertrophy (LVH), because the prevalence of this phenotype is relatively high and is associated with an increased risk of cardiovascular morbidity and mortality [4,5].