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Review Article

Involvement of the Intrarenal Renin-Angiotensin System in Experimental Models of Glomerulonephritis

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The intrarenal renin-angiotensin system (RAS) has several pathophysiologic functions not only in blood pressure regulation but also in the development of glomerulonephritis (GN). Angiotensin II (Ang II) is the biologically active product of the RAS. Locally produced Ang II induces inflammation, renal cell growth, mitogenesis, apoptosis, migration, and differentiation, regulates the gene expression of bioactive substances, and activates multiple intracellular signaling pathways, leading to tissue damage. Activation of the Ang II type 1 (AT1) receptor pathway results in the production of proinflammatory mediators, cell proliferation, and extracellular matrix synthesis, which facilitates glomerular injury. Previous studies have shown that angiotensin-converting enzyme inhibitors and/or AT1 receptor blockers have beneficial effects in experimental GN models and humans with various types of GN, and that these effects are more significant than their suppressive effects on blood pressure. In this paper, we focus on intrarenal RAS activation in the pathophysiology of experimental models of GN.

1. Introduction

The role of the renin-angiotensin system (RAS) in blood pressure regulation and sodium and fluid homeostasis is well recognized [1, 2]. The biologically active peptides that are formed from angiotensinogen (AGT) include angiotensin II (Ang II) and Ang 1-7. The balance between the vasoconstricting actions of Ang II, which are mediated by the Ang II type 1 (AT1) receptor, are countered by the vasodilating actions of Ang II, which are mediated by the AT2 receptor [3] and the actions of Ang 1-7 on the G protein-coupled receptor Mas [4]. Formation of Ang II is dependent upon the substrate availability of AGT and Ang I and the activities of renin, angiotensin-converting enzyme (ACE), ACE2, and ACE-dependent enzymatic pathways, including serine proteases, tonin, cathepsin G, trypsin, and kallikrein. The actions of Ang II are determined by signaling via AT1 and AT2 receptors and the putative Ang 1-7 receptor Mas [5].

Local/tissue RAS in specific tissues has become the focus of much recent interest [6]. Emerging evidence has demonstrated the importance of tissue-specific RAS in the brain [7], heart [8], adrenal glands [9], vasculature [10, 11], and the kidneys [12]. Renal RAS in particular is unique, because all of the components necessary to generate intrarenal Ang II are present along the nephron in both interstitial and intratubular compartments [2, 5]. AGT is the only known substrate for renin that is a rate-limiting enzyme of the RAS. Because the level of AGT is close to the Michaelis-Menten constant for renin, not only renin levels but also AGT levels can control RAS activity, and AGT upregulation may lead to elevated angiotensin protein levels and increased blood pressure [13]. Recent studies have shown that AGT plays an important role in the development and progression of hypertension and kidney disease [2, 12]. Renin mRNA and renin-like activity have been observed in cultured proximal tubular cells [14]. The brush border membrane of proximal human kidney tubules also expresses abundant levels of ACE, mRNA [15], and protein [16]. ACE has been measured in proximal and distal tubular fluid, with greater concentrations observed in proximal tubule fluid [17]. Therefore, all the major components required to generate Ang II are expressed within the kidneys [2, 12].

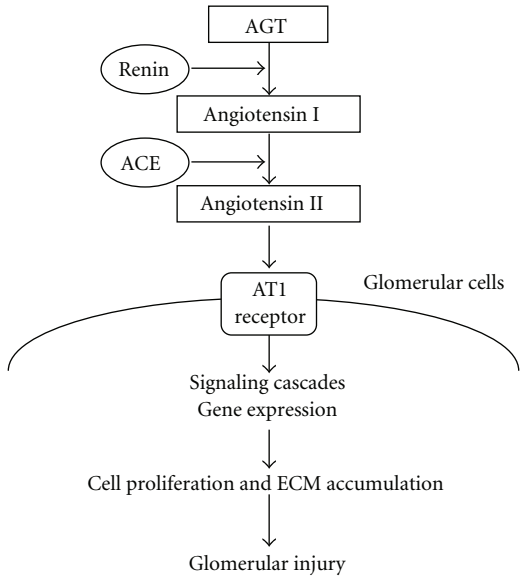

Figure 1: Working schematic of the renin-angiotensin system in glomerulonephritis. AGT: angiotensinogen, ACE: angiotensin-converting enzyme, AT1 receptor: angiotensin II type 1 receptor, and ECM: extracellular matrix.

Chronic glomerulonephritis (GN) resulting in substantial renal damage is frequently characterized by relentless progression to end-stage renal disease. Renal Ang II, the production of which is enhanced in chronic GN, can elevate intraglomerular pressure, increase glomerular cell hypertrophy, and augment extracellular matrix (ECM) accumulation [18, 19] (Figure 1). ACE inhibitors and/or AT1 receptor blockers (ARBs) are often administered to patients with proteinuric nephropathies [20, 21]. The efficacy of these agents in this indication suggests that factors other than Ang II play an important role in the progression of renal disease. This paper explores recent findings concerning the involvement of intrarenal RAS activation in experimental models of GN.

2. RAS Activation in a Model of Progressive Mesangioproliferative GN

Anti-Thy-1 antibody-induced GN is the most common model of experimental nephritis [22, 23], because selective damage to mesangial cells (MCs) allows for the study of mesangial function and pathophysiology. This antibody-antigen reaction initiates complement activation to form a membrane attack complex. Repeated anti-Thy-1 antibody injections may produce progressive glomerular lesions ending in sclerosis, resembling human progressive GN [24]. Glomerulosclerosis is characterized by a continuative accumulation of the ECM, due to increased synthesis and decreased degeneration of the ECM, and overproduction of transforming growth factor (TGF)-β1 in the glomerulus. Furthermore, uninephrectomized rats treated with a single injection of the anti-Thy-1 antibody develop hypertension, massive proteinuria, and severe glomerular injury, finally resulting in chronic mesangioproliferative glomerulosclerosis [25].

To elucidate the involvement of intrarenal RAS activation in the development of glomerulosclerosis during the course of anti-Thy-1 GN with uninephrectomy, we performed an interventional study using the ARB candesartan in a rat model of progressive mesangioproliferative GN [26]. Reactive oxygen species (ROS) produced by NADPH oxidase have been implicated in the development and progression of GN [27, 28]. Ang II induces the activation of NADPH oxidase and the development of oxidative stress in GN [29]. We therefore also examined whether the interaction between the RAS and ROS is important for the development of progressive GN. Nephritis was induced in rats by a single intravenous injection of 2 mg of the nephritogenic anti-Thy-1 antibody 1 week after uninephrectomy. Rats were divided into 4 groups and administered daily oral doses of (1) vehicle, (2) 1% probucol, a free radical scavenger, (3) 70 mg/L candesartan in drinking water, or (4) probucol plus candesartan. Rats in each group were killed at 56 days after the injection of anti-Thy1-1 antibody. As controls, rats were injected with phosphate-buffered saline 1 week after unilateral nephrectomy and were killed on day 56. The ARB candesartan was found to considerably reduce proteinuria, the level of TGF-β1, and ECM accumulation, finally inhibiting the progression of glomerulosclerosis. The combination of probucol and candesartan not only completely eliminated NADPH oxidase components and superoxide production, but also normalized urinary protein excretion and TGF-β1 expression and prevented ECM accumulation, resulting in full prevention of the progression of GN. It seems likely that the beneficial effect of such combined treatment is due to the synergistic action of Ang II inhibition with a receptor antagonist and the elimination of ROS with a radical scavenger. These findings suggest that RAS activation and NADPH oxidase-associated ROS production may play a pivotal role in the progression of GN.

Hydrogen peroxide-inducible clone-5 (Hic-5) was originally discovered as a gene that is induced by TGF-β1 and hydrogen peroxide (H_2O_2), in a study on the growth-inhibitory functions of TGF-β1 in cellular senescence [30]. Recently, Hic-5 has been shown to function as an adaptor protein in focal adhesions and to be involved in integrin-mediated signal transduction, remodeling of the actin cytoskeleton, and regulation of the cellular phenotype [31–33]. To investigate the significance of Hic-5 expression in GN, we analyzed the changes in Hic-5 expression in a rat model of progressive mesangial proliferative GN and examined the combined effects of an ARB and probucol on Hic-5 expression in GN [34]. Glomerular Hic-5 expression increased in parallel with α-smooth muscle actin (SMA) expression in progressive mesangial proliferative GN. Combined therapy with an ARB and probucol in this model improved the histology and expression of Hic-5 and α-SMA. These results suggest that Hic-5 is involved in changes in the MC phenotype, which produce abnormal ECM remolding in GN.

The mitogen-activated protein kinase (MAPK) signaling pathway is a highly conserved module involved in various

cellular functions, including cell proliferation, cell survival, differentiation, and migration [35]. Extracellular stimuli, such as growth factors and environmental stress, induce sequential activation of the MAPK cascade. At least 4 members of the MAPK family have been identified, namely, extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase, p38, and ERK5 [35]. It was recently reported that ERK1/2 activation occurs in the rat Thy-1 model of mesangioproliferative nephritis, and that blocking of the ERK1/2 pathway results in a significant reduction in MC proliferation in this model [36]. In addition, ERK1/2 activation in human glomerulopathies is associated with cell proliferation, histologic lesions, and renal dysfunction [37]. ERK5-mediated MC growth has been reported to be involved in the pathogenesis of diabetic nephropathy [38]. To further elucidate whether ERK signaling is involved in the pathogenesis of GN, we examined the expression and phosphorylation levels of glomerular ERK signals in progressive models of rat mesangioproliferative GN characterized by MC proliferation and ECM accumulation. In addition, the potential role of ERK signaling in MC-mediated pathologic mesangial remodeling was investigated in cultured MCs [39]. Glomerular alterations in progressive models of rat mesangial proliferative GN were examined on days 3, 7, 14, 28, and 56 after anti-Thy1 antibody injection. Light microscopy revealed that almost all glomeruli exhibited faint MC proliferation on day 3, followed by mesangial matrix expansion on day 7. Thereafter, massive accumulation of mesangial ECM and MC proliferation were the prominent features of nephritic glomeruli on days 28 and 56. Immunostaining of kidneys obtained at different time points revealed that phospho-ERK1/2 expression increased on day 7 during the phase of enhanced MC proliferation and decreased thereafter. On the other hand, phospho-ERK5 was weakly expressed in control glomeruli but dramatically increased in a typical mesangial pattern after 28 and 56 days of GN. Semiquantitative assessment indicated that glomerular phospho-ERK5 expression closely paralleled the accumulation of ECM and collagen type 1, as well as glomerular expression of ROS and Ang II. The in vitro study revealed that H_2O_2 and Ang II each induced ERK5 phosphorylation by cultured MCs. Costimulation with both H_2O_2 and Ang II synergistically increased ERK5 phosphorylation in MCs. Cultured MCs transfected with ERK5-specific small interference RNA showed a significant decrease in H_2O_2 or Ang II-induced cell viability and soluble collagen secretion compared with control cells. Finally, treatment of GN rats with an ARB resulted in a significant decrease in phospho-ERK5 expression and collagen accumulation, accompanied by remarkable histologic improvement. Taken together, these results suggest that MC ERK5 phosphorylation by Ang II or H_2O_2 enhances cell viability and ECM accumulation in chronic GN.

Several studies demonstrated that the MAPK signaling controls cell behaviors via under RAS activation in other experimental GN models [40–42]. In diabetic conditions, high glucose generates ROS as a result of glucose auto-oxidation, metabolism, and formation of advanced glycation end production [43]. All these signaling molecules are involved into MAPK signaling pathways in glomerular cells [44]. High-glucose-induced diabetic complications have been implicated, in part, to the activation of MAPK [44]. Interestingly, the stimulation of Ang II by hyperglycemia or oxidative stress activates the MAPK cascade [45]. These results suggest that high-glucose-induced ROS/MAPK pathway and intrarenal RAS activation play key roles in diabetic nephropathy.

3. RAS Activation in a Model of Crescentic GN

Crescentic GN, also known as antiglomerular basement membrane (anti-GBM) disease or Goodpasture's syndrome, is characterized by the formation and deposition of antibodies on the basement membranes of glomeruli and alveoli [46]. The disease progresses rapidly, and patients present with renal failure, dyspnea, hemoptysis, a sudden decrease in hemoglobin level, pallor, and circulatory disturbances. Understanding the underlying proinflammatory responses may help to facilitate the identification of therapeutic targets for arresting the progression of anti-GBM disease. In Wistar-Kyoto (WKY) rats, the administration of a minute dose of anti-GBM antibodies induces severe proliferative and necrotizing GN with crescent formation [47, 48]. In rat models of anti-GBM disease, glomerular infiltration by T lymphocytes, monocytes/macrophages, and some neutrophils is the earliest and most prominent pathologic change [48]. To investigate whether local RAS activation occurs in nephritic glomeruli with crescent formation and whether the final effector molecule Ang II contributes to the induction of ROS and inflammation, as well as glomerular pathologic alterations, we studied the effects of an ARB on rat anti-GBM antibody-induced GN by evaluating indexes of glomerular RAS activation, oxidative stress, inflammation, and TGF-β1 expression in GN rats [49]. Progressive anti-GBM GN was induced in 7-week-old male WKY rats by a single injection of anti-GBM antiserum. Vehicle-treated nephritic rats showed severe proteinuria and developed crescentic GN accompanied by marked macrophage infiltration and enhanced expression of glomerular α-SMA, AGT, Ang II, AT1 receptor, and NADPH oxidase on day 28. Treatment with an ARB improved proteinuria and pathologic alterations such as crescent formation and glomerulosclerosis, and had a significant inhibitory effect on these parameters on day 28 of GN. Enhanced superoxide production in nephritic glomeruli was also decreased by the ARB. Moreover, Ang II and TGF-β1 in the supernatant of cultured glomeruli was increased significantly in vehicle-treated nephritic rats, whereas the production of these compounds was significantly inhibited in ARB-treated rats on day 28. These findings indicate that increased glomerular RAS activity and the resulting increase in Ang II production plays an important role in progressive glomerular injury by inducing oxidative stress and TGF-β1 expression.

Recent studies have revealed that monocyte chemoattractant protein-1 (MCP-1) is involved in the pathogenesis of crescentic GN [50]. MCP-1 is presumed to be a key mediator of chemotaxis and the activation of macrophages [51]. Chronic Ang II infusion in rats activates MCP-1 and

TGF-β1, which in turn induces macrophage infiltration in renal tissues [52]. CC chemokine receptor 2 (CCR2), a cognate receptor of MCP-1 expressed mainly on monocytes, has been reported to be involved in human crescentic GN [53]. Based on these principles, we hypothesized that therapeutic management of anti-GBM disease may be achieved by blocking the MCP-1/CCR2 signaling pathway and RAS [54]. Whereas treatment with a CCR2 antagonist (CA) or ARB alone only moderately ameliorated kidney injury in a rat model of crescentic GN, combination treatment with a CA and an ARB dramatically prevented proteinuria and markedly reduced glomerular crescent formation. Further, combination treatment suppressed macrophage infiltration, reduced MCP-1, AGT, Ang II, and TGF-β1 expression, and reversed the fibrotic change in glomeruli. Primary cultured glomerular MCs stimulated by Ang II showed significant increases in MCP-1 and TGF-β1 expression. Furthermore, a coculture model consisting of MCs, parietal epithelial cells, and macrophages showed an increase in Ang II-induced cell proliferation and collagen secretion. ARB treatment attenuated these effects. These data suggest that Ang II enhances glomerular crescent formation, and inhibition of the MCP-1/CCR2 pathway with a CA/ARB combination effectively reduces renal injury in anti-GBM nephritis.

Recent reports indicated roles of RAS activation and MCP-1/CCR2 pathway in other nephritis models [55–58]. Most studies concluded that activation of RAS is an important determinant of local MCP-1 expression, either directly or indirectly through glomerular hemodynamic effects. However, whether the manipulation of MCP-1/CCR2 pathway and RAS is beneficial with respect to the progression of human and experimental GN remains to be investigated.

4. Conclusion

The present studies reveal that intrarenal RAS activation has important pathophysiologic functions in the development of progressive mesangioproliferative and crescentic GN. Additional studies are needed to determine the relationship between RAS activation and glomerular injury, such as cell proliferation, sclerosis, and crescent formation, and to clarify the mechanisms underlying Ang II-induced pathologic glomerular changes. Furthermore, it is necessary to determine whether RAS inhibition may provide a clinically significant pharmacologic strategy for the therapeutic treatment of progressive GN.

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