Angiotensinogen Expression Is Enhanced in the Progression of Glomerular Disease

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ABSTRACT

Intrarenal renin-angiotensin system (RAS) activation plays a critical role in the development and progression of renal injury. In the kidney, all of the RAS components are present and intrarenal angiotensin II (Ang II) is formed by multiple independent mechanisms. Angiotensinogen (AGT) is the only known substrate for renin that is a rate-limiting enzyme of the RAS. Recently, enhanced intrarenal AGT levels have been shown to reflect the intrarenal RAS status in hypertension, chronic glomerular disease and diabetic nephropathy. In this review, we focus on AGT expression of the diseased glomeruli in the progression of glomerular disease. An anti-glomerular basement membrane nephritis rat model developed progressive proteinuria and glomerular crescent formation, accompanied by increased macrophage infiltration and glomerular expression of AGT and Ang II. The addition of Ang II type 1 receptor blocker to CC-chemokine receptor 2 antagonist markedly attenuated the induction of macrophage infiltration, AGT and Ang II, and reduced glomerular crescent formation. Next, the levels of glomerular AGT expression and marker of reactive oxygen species in Zucker diabetic fatty (ZDF) obese rats were higher than those in ZDF lean rats. Hydrogen peroxide (H$_2$O$_2$) induced an increase in the AGT expression in primary rat mesangial cells. Furthermore, the H$_2$O$_2$-induced upregulation of AGT was inhibited by a mitogen-activated protein kinase kinase and an c-Jun N-terminal kinase inhibitor. These data suggest the potential contribution of enhanced AGT expression in glomeruli to the intrarenal RAS activation for the development of glomerular disease.

Keywords: Renin-Angiotensin System, Angiotensinogen, Glomerulonephritis, Glomerulosclerosis

1. Introduction

The critical role of the renin-angiotensin system (RAS) in arterial pressure and sodium homeostasis has been widely recognized [1,2]. Angiotensin II (Ang II) is the most powerful biologically active product of the RAS [3]. Ang II directly constricts vascular smooth muscle cells, enhances myocardial contractility, stimulates aldosterone production, stimulates release of catecholamines from the adrenal medulla and sympathetic nerve endings, increases sympathetic nervous system activity, and stimulates thirst and salt appetite [3]. Recently, the focus of interest in the RAS has shifted toward the role of the local/tissue RAS in specific tissues [4]. Locally produced Ang II induces inflammation, cell growth, mitogenesis, apoptosis, migration, and differentiation; regulates the gene expression of bioactive substances; and activates multiple intracellular signaling pathways, all of which might contribute to tissue injury [3]. Intrarenal RAS activity has several pathophysiological functions for not only in blood pressure regulation but also in renal cell growth and production of glomerulosclerosis, which contributes to the development of renal fibrosis [5,6]. Indeed, previous studies have shown that angiotensin converting enzyme inhibitor (ACEi) and/or Ang II type 1 (AT1) receptor blocker (ARB) have beneficial effects in rats and humans with various renal diseases, and these effects are often considerably more significant than their suppressive effects on blood pressure [7,8].

Many diseases affect kidney function by attacking the glomeruli—the tiny units within the kidney in which blood is filtered [9]. Glomerular diseases include many conditions with a variety of genetic and environmental causes, but they fall into 2 major categories: glomerulonephritis and glomerulosclerosis. Although glomerulonephritis and glomerulosclerosis have different causes, they can both lead to kidney failure [9]. Activation of the Ang II-AT1 receptor pathway results in production of...
proinflammatory mediators, cell proliferation, and extracellular matrix synthesis that facilitate kidney damage and advance chronic kidney disease [10-13] (Figure 1). This review explores recent findings concerning the expression of angiotensinogen (AGT) in the intrarenal RAS activation in glomerular disease.

2. Intrarenal RAS

The biologically active peptides that are formed from AGT include Ang II and angiotensin-(1-7). The balance between the vasoconstricting actions of Ang II, mediated by the AT \(_1\) receptor, are countered by the vasodilating actions of Ang II, mediated by the AT \(_2\) receptor [14], and the action of angiotensin-(1-7) on the G protein-coupled receptor Mas [15].

Local/tissue RAS in specific tissues has become the focus of much recent interest [4]. Emerging evidence has demonstrated the importance of tissue-specific RAS in the brain [16], heart [17], adrenal glands [18], and vasculature [19] as well as the kidneys [20]. In particular, renal RAS is unique because all of the components necessary to generate intrarenal Ang II are present along the nephron in both interstitial and intratubular compartments [3,21]. The presence of AGT gene transcription in the proximal tubules has been shown using in situ hybridization [22]. AGT mRNA is expressed primarily in the proximal convoluted tubules and proximal straight tubules, with small amounts in glomeruli, vasa recta, and renal vasculature [23]. Renal AGT protein is abundant in the proximal convoluted tubules [24,25]. Strong positive immunostaining for AGT protein has been reported in proximal convoluted tubules and proximal straight tubules, and weak positive staining in glomeruli and vasa recta; however, distal tubules and collecting ducts were negative [26]. The AGT synthesized in the kidney is secreted into the lumen leading to Ang I generation. Low but measureable renin concentrations have been detected in proximal tubule fluid in rats [27].

Renin mRNA and renin-like activity have been demonstrated in cultured proximal tubular cells [28-30]. The brush border membrane of proximal human kidney tubules expresses abundant levels of ACE mRNA [31] and protein [32,33]. ACE has also been measured in proximal and distal tubular fluid but is greater in proximal tubule fluid [34]. Therefore, all of the major components required to generate Ang II are expressed within the kidney [3,20].

3. AGT in Intrarenal RAS Activation

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 peptide levels and increased blood pressure [35,36]. Recent studies using experimental animal models and transgenic mice have documented AGT involvement in the activation of the RAS and development of hypertension [37-45]. Genetic manipulations that lead to AGT overexpression have consistently been shown to cause hypertension [46,47]. In human genetic studies, a linkage has been established between the AGT gene and hypertension [48-51]. Enhanced intrarenal AGT mRNA and/or protein levels have also been observed in multiple experimental models of hypertension including Ang II-dependent hypertensive rats [26,52-56], Dahl salt-sensitive hypertensive rats [57,58], and spontaneously hypertensive rats [59], as well as in kidney diseases including diabetic nephropathy [60-65], IgA nephropathy [66,67], and radiation nephropathy [68]. In addition, a direct quantitative method to measure urinary AGT using human AGT enzyme-linked immunosorbent assays (ELISA) was developed [69], which revealed significantly increased urinary AGT levels in patients with hypertension [70,71], chronic kidney disease [72-75] and diabetes [76, 77]. Thus, AGT plays an important role in the development and progression of hypertension and kidney disease [3,20].

4. AGT in Anti-Glomerular Basement Membrane (GBM) Disease

Chronic glomerulonephritis that results 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 glomerulonephritis, can elevate the intraglomerular pressure, increase glomerular cell hypertrophy, and augmented extracellular matrix accumulation [78,79]. ACEi and/or ARB markedly decelerate, and can even prevent, renal deterioration in renal disease [1,78,80,81]. This may reflect the relatively short-term nature and small size of these studies, but may also be an indication that factors other than Ang II play an important role in the progression of renal disease.

Anti-GBM disease or Goodpasture’s syndrome is a crescentic glomerulonephritis that is characterized by the formation and deposition of antibodies on the basement membranes of glomeruli and alveoli [82]. Patients present with renal failure, dyspnea, hemoptysis, a sudden decrease in the hemoglobin level, pallor, and circulatory disturbances. Most patients with advanced disease do not respond to plasmapheresis or immunosuppression therapy [83]. While kidney transplantation is an option, a patient should wait for 6 months or after the disappearance of serum anti-GBM antibodies before undergoing kidney transplantation because of the risk of recurrence [82]. Therefore, a novel therapeutic strategy is needed. Studies based on anti-GBM antibody have focused on elucidating the molecular and cellular mechanisms involved in the pathogenesis of this disease. Understanding the mechanisms of proinflammatory responses help facilitate the identification of therapeutic targets that arrest the progression of anti-GBM disease.

Glomerular crescents are defined as the presence of 2 or more layers of cells in the Bowman’s space. Monocyte/macrophages and parietal epithelial cells are the principal mediators of crescent formation [84]. The presence of crescents in glomeruli is a marker of severe injury [90]. After a single injection of anti-GBM antibodies, marked crescent formations were observed in almost all glomeruli as a result of severe glomerular damage. CCR2 antagonist (CA) or ARB alone moderately normalized the crescent formation [91]. The dose of CA or ARB was determined by previous reports [92,93] and could be adequate to preclude the effects of the MCP-1/CCR2 signal pathway and RAS. Their combination significantly blocked the development of crescent formation, preventing the infiltration of macrophages [91]. Consistently, the combination therapy markedly reduced proteinuria.

It is well known that intrarenal RAS activation is a major mediator of progressive renal injury in glomerulonephritis [78-81]. In this anti-GBM disease model, the glomerular expression levels of AGT, Ang II and AT1 receptors were increased compared with control rats [91]. While CA or ARB treatment moderately reduced the increase of these components in glomeruli, CA plus ARB treatment further prevented these increases (Figures 2(a)-(d)). Urinary AGT levels were paralleled with the expression levels of RAS components in glomeruli (Figure 2(e)). The disturbance in the expression of these components likely plays an important role in the pathogenesis of the crescentic formation in glomerulonephritis. Furthermore, Ang II reportedly upregulated AGT and Ang II receptor expressions and ARB prevented the increase of AGT, suggesting positive Ang II feedback in the kidney [94]. Interestingly, ARB treatment prevented increases in kidney and renal interstitial fluid Ang II concentration in the Ang II-infused rat [95]. Thus, from these findings, combination therapy suppressed these expressions more effectively than did CA or ARB alone, cutting intrarenal RAS activation. In previous studies, the RAS activation was shown to be involved in the formation of glomerular crescents [96,97]. Together, these data clearly indicate that blocking the RAS is a key target and that AGT expression reflects intrarenal RAS activation in treatment of anti-GBM disease.

5. AGT in Diabetic Nephropathy

Glomerulosclerosis refers to scarring of the glomeruli. In several sclerotic conditions, a systemic disease such as diabetes is responsible. Glomerulosclerosis is caused by the activation of glomerular cells to produce scar material. This may be stimulated by molecules called growth factors, which may be made by glomerular cells themselves or may be brought to the glomerulus by circulating blood that enters the glomerular filter.

Diabetic nephropathy is the most common etiology of end-stage renal failure in patients starting dialysis [98]. A pathological feature of diabetic nephropathy is the thickening of the GBM and expansion of the mesangium due to accumulation of extracellular matrix [99]. The detailed mechanisms responsible for the development and progression of diabetic nephropathy have yet to be fully elucidated. However, the renoprotective effects of drugs that block or interfere with the RAS indicate that inappropriate activation of the RAS contributes to diabetic nephropathy [100,101]. ACEi reduces proteinuria in patients with diabetes [100]. In a large-scale clinical study, captopril, an ACEi, provided protection against deterioration of renal function in diabetic nephropathy patients with type 1 diabetes [101]. Similarly, losartan, an ARB, conferred significant renal benefits in diabetic nephropathy patients with type 2 diabetes [102]. More recently, olmesartan, another ARB, has been shown to suppress the incidence of microalbuminuria in patients with type 2 diabetes [103,104]. Thus, the renoprotective effects of ACEi and ARBs have been established in various studies. Combination therapy with ACEi and ARBs provides greater renoprotection than with ACEi alone in diabetic renal disease [105,106] suggesting that ACE-independent
Figure 2. Effects of the treatments on intrarenal AGT, Ang II, and AT1 receptor (AT1R) expression and urinary AGT expression in an anti-GBM disease rat model. (a) AGT (top), Ang II (middle), and AT1R (bottom) immnostained kidney sections. Densitometric analyses of AGT (b), Ang II (c) or AT1R (d) expressions determined by immunostaining. Data are mean +/- SEM. *P < 0.05 (vs. vehicle); **P < 0.01 (vs. vehicle); ***P < 0.001 (vs. vehicle).
pathways for Ang II formation may be of significance.

The Zucker diabetic fatty (ZDF) obese rat is considered to be an excellent animal model of type 2 diabetes because it presents a physiological and metabolic profile similar to that seen in humans [107,108]. The ZDF obese rat is characterized by hyperglycemia, hyperinsulinemia, hyperlipidemia, moderate hypertension, moderate obesity and progressive renal injury [109,110]. The ZDF obese rat exhibits progressive diabetic nephropathy at approximately 20 weeks of age [107]. Mizuno et al. [108] demonstrated that pharmacological blockade of the RAS with an ARB reduced proteinuria and delayed the progression of renal disease in diabetic nephropathy in ZDF obese rats. This suggests that the activated intrarenal RAS plays an important role in the development of diabetic nephropathy in ZDF obese rats. Meanwhile, previous studies revealed that the reactive oxygen species (ROS)-related increase in AGT plays an important role in the development of renal injury in genetic salt-sensitive hypertension [57,58]. Moreover, it has been recently reported that the activated intrarenal ROS-AGT axis plays a role in the development of IgA nephropathy in patients at an early stage [66].

Therefore, ZDF obese rats were examined in terms of enhanced ROS-associated augmentation of intrarenal AGT was involved in the development of nephropathy. As a result, the levels of glomerular immunoreactivity for 4-HNE and urinary excretion of 8-isoprostane, a marker of ROS-in ZDF obese rats were higher than those in ZDF lean rats [64,111]. The levels of glomerular AGT immunoreactivity and in ZDF obese rats were higher than those in ZDF lean rats. Double staining with AGT and Thy1.1 antibodies showed that the majority of AGT in glomeruli was seen in mesangial cells [111]. Relative ratios of intrarenal Ang II immunoreactivity were significantly increased in ZDF obese rat glomeruli compared with controls [64]. These data suggest that the sequential activation of the ROS-AGT-RAS axis plays an important role in the development of diabetic nephropathy in ZDF obese rats.

The signal transduction pathways involved in AGT expression are currently being investigated. Recent studies showed that in immortalized human renal proximal tubular epithelial cells, Ang II acts synergistically with interleukin-6 to increased AGT expression through activation of nuclear factor-kB and the signal transducer and activator of transcription-3 [112]. It has also been reported that in immortalized rat proximal tubular cells, a high glucose concentration stimulates AGT expression through ROS generation and subsequent p38 mitogen-activated protein kinase (MAPK) expression [113]. However, the signal transduction pathway that induces AGT expression has not yet been completely elucidated yet. To clarify the signal transduction pathway for glomerular AGT expression, primary rat mesangial cells were treated with hydrogen peroxide (H$_2$O$_2$) [111]. H$_2$O$_2$ induced an increase in AGT expression in a dose- and time-dependent manner, and the H$_2$O$_2$-induced upregulation of AGT was suppressed by catalase. Furthermore, the H$_2$O$_2$-induced upregulation of AGT was inhibited by a MAPK kinase (MEK) inhibitor and a c-Jun N-terminal kinase (JNK) inhibitor, but not inhibited by a p-38 MAPK inhibitor.

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6. Conclusions

This review reveals that AGT expression is enhanced in the glomerular injury associated with RAS activation. Additional studies are needed to clarify the mechanism for AGT overexpression by intrarenal RAS activation in glomerular disease. Intrarenal RAS activation clearly plays a pivotal role in the development of glomerular disease. Accordingly, the assessment of glomerular AGT expression may be of substantial importance. We believe that the investigation of AGT expression in the glomeruli could provide a novel pharmacological strategy for the treatment of glomerular disease.

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