Renal Interstitial Fibrosis and Angiotensin Inhibition

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Tubulointerstitial (TI) fibrosis is a final common pathway to progressive renal injury of all forms of renal disease. However, once renal damage reaches a certain threshold, progression of renal disease is consistent, irreversible, and largely independent of the initial injury. Angiotensin (AT) II is the main effector of the renin angiotensin system (RAS) and effects that may contribute to the onset and progression of renal damage. AT II may also directly contribute to accelerate renal damage by sustaining cell growth, inflammation, and fibrosis. Interventions that inhibit the activity of the RAS are renoprotective and may retard or even halt the progression of chronic nephropathics. Unilateral ureteral obstruction suggested as a well-established experimental model of progressive interstitial expansion and fibrosis. Although technically challenging, some investigators have successfully relieved the obstruction and reported significant reduction in interstitial fibrosis severity. Drugs that modulate the RAS, such as ACE inhibitors and angiotensin type 1 (AT1) receptor antagonists, have demonstrated protective renal effects and can ameliorate fibrosis. However, neither ACE inhibitor nor AT1 receptor blockade completely suppresses progression of renal disease. Dual blockade of the RAS with ACE inhibitors and AT1 receptor blockers may provide renal benefit beyond therapy with either drug alone, due to their potential additive beneficial effect.

Key Words : Fibrosis, Unilateral ureteral obstruction, Angiotensin inhibition

Tubulointerstitial (TI) fibrosis is a common feature of progressive renal injury in almost all forms of renal diseases. It has been shown that TI injury is a more consistent predictor of functional impairment than glomerular damage[1, 2]. Chronic inflammation generally precedes the development of fibrosis and inflammatory cytokines are important mediators of fibrogenesis. Patients with TI fibrosis have a rather poor prognosis and often progress to end-stage renal failure[3]. About 80% of total kidney volume is composed of tubular epithelial cells and cells within the interstitial space. Renal tubular epithelial cells represent the major cellular compartment of the kidney. The interstitium is surrounded by vascular and tubular compartments, and its communication with the glomerular and extraglomerular mesangium makes it especially vulnerable to pathologic events originating in these neighboring areas. Structural derangements of the TI compartment occur in virtually all progressive renal diseases[4].

An expansion of the cortical interstitium is highly correlated with tubular lesions, especially tubular atrophy. The widening of the interstitial space in chronic renal diseases is mainly due to increased extracellular matrix (ECM), and increased cellularity (fibroblasts, macrophages, and lymphocytes) may also contribute to the tubulointerstitial fibrosis[5-7]. However, once renal damage reaches a certain threshold, progression of renal disease is consistent, irreversible, and largely independent of the initial injury.

The renin angiotensin system (RAS) is a well-known regulator of blood pressure (BP) and determinant of target organ damage. It controls fluid and electrolyte balance through coordinated effects on the heart, blood vessels and kidneys. Angiotensin (AT) II is the main effector of the RAS and exerts its vaso-

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constrictor effect predominantly on the postglomerular arterioles, thereby increasing the glomerular hydraulic pressure and the ultrafiltration of plasma proteins, effects that may contribute to the onset and progression of chronic renal damage. AT II may also directly contribute to accelerate renal damage by sustaining cell growth, inflammation, and fibrosis. Interventions that inhibit the activity of the RAS are renoprotective and may retard or even halt the progression of chronic nephropathies[8].

Renal injury and repair comprises a delicate balance between cell loss and proliferation and ECM accumulation and remodeling[9]. In rodents complete ureteral obstruction induces aggressive interstitial fibrosis and tubular atrophy. Although technically challenging, some investigators have successfully relieved the obstruction and reported significant reduction in interstitial fibrosis severity[10]. So it is possible to investigate and clarify the mechanism of renal repair or remodeling after renal fibrosis.

Tubulointerstitial fibrosis

Tubules and interstitium make up approximately 80% of the renal volume, and occupy the compartment referred to as the tubulointerstitium. The interstitium space takes up 10% of rat and up to 17% of dog and rabbit kidneys, including 7 to 9% of renal cortex, 3 to 5% of outer medulla and 30 to 40% of inner medulla and papillary tip[10-13]. It is made up of both cellular and matrix components. The matrix is made up of a fibrillar net of interstitial and basement membrane collagens and associated proteoglycans, glycoproteins and interstitial fluid. The interstitial compartment not only provides structural support for the individual nephrons, but also serves as a conduit for solute transport[12]. It is also the site of production of several hormones and cytokines such as erythropoietin and prostaglandins. Renal fibrosis is pathologically characterized by interstitial fibrosis, tubular atrophy, capillary loss, and podocyte depletion. In most chronic kidney diseases there is an intimate relationship between interstitial fibrosis and tubular atrophy, both of which are closely correlated with the decline in renal function1[4]. Careful pathological analysis revealed that the impairment of renal function correlates better with the extent of TI damage than with the degree of glomerular damage[2]. It might be possible to assume that interstitial fibrosis causes tubular atrophy, although this is difficult to prove. The underlying cellular events leading to these histologic presentations are even more complicated; they include mesangial and fibroblast activation, tubular epithelial to mesenchymal transition (EMT), monocyte/macrophage and T-cell infiltration, and cell apoptosis[14, 15].

In renal fibrosis, a balance between the matrix synthesis and matrix degradation is changed due to increased matrix synthesis or decreased degradation[3]. Renal fibrogenesis may be divided into three phases: induction, matrix synthesis, and resolution[6]. The induction phase is characterized by the promotion of proliferation and motility of fibroblasts. In the phase of matrix synthesis, several interstitial matrix proteins such as collagen type I, III, fibronectin and various proteoglycans are produced and deposited in the renal interstitium. Final resolution phase is characterized by the cessation of inflammatory reactions and matrix production. The biological process of renal fibrosis is essentially a wound-healing response. Ideally this process should result in the regeneration of normal tissue. Therefore, fibrosis can be defined as pathological response caused by the chronic and/or severe inflammatory stimuli. Individual cells, especially tubular cells, can regenerate by proliferation following acute injury, but more advanced disease that is associated with damaged tubular basement membranes and tubular atrophy appears to be irreversible[3, 16]. After the initial injury, the affected kidney tissues undergo a series of events in an attempt to repair and recover from the damage. These processes include kidney resident cell activation, which leads to the production and secretion of proinflammatory cytokines. The gradients of che-

motactic cytokines provide a directional signal for guiding the infiltration of inflammatory monocytes/macrophages and T cells to the injured sites. Depending on the etiology of renal injury, glomerular or interstitial infiltrated inflammatory cells become activated, and produce injurious molecules such as reactive oxygen species, as well as fibrogenic and inflammatory cytokines[14].

TI injury is mediated by massive proteinuria containing a large amount of complement components and chronic hypoxia with loss of peritubular capillaries in the tubulointerstitium[17]. Proteinuria may contribute to tubulointerstitial damage by activation of nuclear factor-kappa B (NF-κB) and upregulation of various proinflammatory and profibrotic genes. Inhibition of NF-κB improved TI injury and is also associated with the transdifferentiation of tubular cells into myofibroblasts[17]. Hypoxia can activate fibroblasts, change ECM metabolism of resident renal cells, and lead to eventual fibrogenesis[17].

Unilateral ureteral obstruction study for renal interstitial fibrosis

In the study of human diabetic glomerular disease by Fioretto et al[18], once diabetes was cured by successful pancreas transplantation, it took 10 years for the expanded mesangial matrix to regress.

However, diabetic renal changes in man are very slow to develop and to reverse and possible manipulations of this human model are limited by ethical constraints. Therefore, unilateral ureteral obstruction (UUO) model in the rat can be used for the exploration of these phenomena rapidly. UUO represents a well-established experimental model of progressive interstitial expansion and fibrosis[19, 20]. UUO causes rapid interstitial expansion and fibrosis and tubular injury[19, 20]. In the rabbit, interstitial fibrosis is seen in the affected kidney at 7 days (D) of UUO. Moreover, increased synthesis of ECM components [collagens I, III, and IV, fibronectin and heparan sulfate proteoglycan] is detectable at 3-7D of UUO[19]. The expression of transforming growth factor (TGF)-β in rat kidneys after 1 and 7D of UUO is also increased in the interstitium and in perivascular areas[20]. These alterations are remarkably alleviated by prior or concomitant use of an angiotensin converting enzyme (ACE) inhibitor and a specific angiotensin II receptor blocker[19-21]. These data suggest that interstitial fibrosis may begin promptly after the onset of obstruction and partially may result from enhanced expression of TGF-β which in turn may be regulated by local AT II generation. Recovery of nephron function after release of UUO in rats is highly dependent on the duration of obstruction, and the extent of interstitial fibrosis.

Early relief of the obstruction is the therapy of choice, however this is not always feasible. Thus, other treatments need to be developed to help halt or reverse renal injury consequent to obstructive nephropathy. This maneuver may be helpful to retard or even reverse the injury developed.

A number of cytokines, vasoactive compounds, chemoattractant molecules, and growth factors are upregulated after the onset of obstructive uropathy, and the RAS is activated after ureteral obstruction[22, 23]. Increasing levels of AT II might in turn upregulate the expression of other factors, such as TGF-β1, tumor necrosis factor α (TNF α), osteopontin, and NF-κB. TGF-β1 affects a wide variety of proteins found in the ECM including : fibronectin; collagen types I, II, III, IV, and V and proteoglycans. TGF-β1 inhibits matrix degradation by increasing the activity of tissue inhibitors of metalloproteinases and decreasing the activity of metalloproteinases. It also stimulates the synthesis of receptors for ECM proteins. Furthermore, TGF-β1 is a chemoattractant for fibroblasts and stimulates fibroblast proliferation[22, 23]. Thus, this cytokine likely contributes importantly to the accumulation of ECM protein in the renal interstitium. Several cytokines might initiate fibrogenesis, but TGF-β1 is considered a major stimulating factor.

In the animal experiment[10] for remodeling of renal interstitium after release of complete UUO, release of UUO lead to 20% reduction of the interstitial expansion but 80% of the increase remained. With ACE inhibitor (enalapril) the decrease in the interstitial expansion was nearly 34%, significantly faster than with release alone. Unfortunately, complete recovery of interstitial damage developed after UUO could not be reached even after the treatment of ACE inhibitor. It is known this beneficial effect is independent of BP lowering of ACE inhibitor and is more likely due to influences on ongoing RAS activation. Reversal of interstitial fibrosis occurred when ureteral obstruction was released in neonatal rats, but not to the preobstruction level[24]. This partial reversal was accompanied by persistently abnormal expression of growth factors such as TGF-β, epidermal growth factor and continuous activation of the RAS. This persistent excess generation of AT II, may explain the additional beneficial effect of ACE inhibitor in ameliorating interstitial fibrosis, after the release of ureteral obstruction[10, 24]. ACE inhibitor may accelerate healing process through yet to be described mechanisms but probably not through anti-inflammatory effects.

Hemodynamic independent action of renin angiotensin system

The classical role of the RAS has been to serve as a vasoconstrictor and to facilitate renal sodium absorption, serving to maintain BP. Inhibition of RAS (ACE inhibitor or angiotensin type 1 receptor blocker) has an effect to decrease of the intraglomerular pressure. AT II, the main peptide of the 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 ECM 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. Blockade of AT II actions by ACE inhibitors and angiotensin type 1 (AT_1) receptor antagonists prevents proteinuria, gene expression upregulation, and fibrosis, as well as inflammatory cell infiltration. Interestingly, AT 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 : AT II (1) activates mononuclear cells and (2) increases proinflammatory mediators (cytokines, chemokines, adhesion molecules, NF-κB). Finally, AT 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[25].

AT II is a chemotactic factor for inflammatory cells and increases chemokines and adhesion molecules in both resident and infiltrating cells. This inflammatory response could be direct, by MCP-1 and TGF-β production, and indirect, by activation of resident cells by macrophage-related factors, and therefore contributes to the progression of fibrosis. In renal cells, AT II increases several proteins involved in cell growth and matrix regulation. Proteinuria could activate renal RAS and therefore may participate in renal injury[25]. AT II may promote the phenotypic change of fibroblasts to myofibroblasts. These activated fibroblasts may proliferate and invade the periglomerular and peritubular spaces, contributing to matrix deposition in the TI area. Indeed, rats chronically infused with AT II develop injury with tubular atrophy and dilation, cast formation, interstitial monocyte infiltration, and interstitial fibrosis with type IV collagen deposition[26]. Cultured renal interstitial fibroblasts express AT_1 receptors, and after AT II stimulation, there is an increase in cell proliferation and expression and synthesis of ECM proteins, such as fibronectin, via a TGF-β-mediated mechanism[27].

Renal tubular cells also play a central role in the pathogenesis of interstitial fibrosis. Tubular cells might

be stimulated by the spill over of peptides such as AT II, growth factors, and cytokines from the injured glomeruli. In cultured tubular cells, AT II causes hypertrophy and also increases the synthesis of ECM via TGF-β[28].

Emerging evidence suggests that connective tissue growth factor (CTGF), a novel profibrogenic cytokine, may play a role in the development and progression of renal fibrosis as a mediator of TGF-β profibrotic activities[29]. Recent experimental data showed that AT II increased CTGF mRNA expression and synthesis and that ACE inhibition diminished renal CTGF expression correlated with diminution of TGF β and fibrosis. These results suggest that CTGF could be a mediator of the matrix accumulation caused by AT II[25, 29].

In using of ACE inhibitor, inhibition of ACE activity decreases the formation of AT II and potentiates the vasodilatory effects of bradykinin. As a result, ACE inhibitors are used widely to treat hypertension. These agents reduce proteinuria and delay progression of renal disease in patients with diabetic nephropathy or nondiabetic kidney disease. AT1 receptor antagonists are also effective antihypertensive agents[30, 31]. Antagonism of the AT1 receptor and binding of AT II to the AT_2 receptor probably underlies their effect. In contrast to ACE inhibitor, AT_1 receptor antagonist (AT_1RA) does not inhibit the breakdown of bradykinin[31]. Clinical trials suggest that AT1RAs reduce microalbuminuria and proteinuria and retard the progression of diabetic and nondiabetic kidney disease, which is similar to ACE inhibitors[31, 32].

Renoprotective benefits of renin angiotensin system inhibition

AT II, the main peptide of the RAS, is involved in the pathogenesis of renal diseases[27]. This peptide acts through its binding to two specific receptors, angiotensin II type 1 (AT_1) and angiotensin II type 2 (AT_2) receptors[33]. In adult tissues, the AT_1 receptor is distributed in the vasculature, kidney, adrenal gland, heart, liver, and brain[34]. In healthy adults, the AT_2 receptor is present only in the adrenal medulla, uterus, ovary, vascular endothelium, and distinct brain areas[34]. The AT_2 receptor is also widely distributed in the fetus. Both AT_1 and AT_2 receptors are developmentally regulated, but the AT_2 receptor is much more abundant in the fetal kidney. Throughout nephrogenesis, the AT_2 receptor is mainly expressed in undifferentiated mesenchyme. It is down-regulated after birth. AT_1 receptors are initially localized in the nephrogenic cortex and developing glomeruli, proximal tubules, and vessels. During maturation of the kidney, AT_1 receptor expression becomes more abundant.

AT_1 is responsible for most of the pathophysiological actions of AT II. By promoting proliferation, inflammation and fibrosis, AT II contributes to chronic diseases, such as hypertension, atherosclerosis, cardiac hypertrophy and renal injury. The role of the AT_2 receptor is not completely defined. The AT_1 receptor mediates the more deleterious effects of AT II - that is, vasoconstriction and cardiac and vessel hypertrophy. In addition to the conversion of AT I to AT II, ACE inactivates the vasodilator peptide bradykinin[35]. AT II mediates both hemodynamic changes and renal growth suggesting that these processes are intimately connected. Both are thought to contribute to progression[36]. The nonhemodynamic actions of AT II include stimulation of tubular transport, facilitation of proteinuria by modifying structure and function of the glomerular ultrafiltration barrier, induction of proinflammatory as well as profibrogenic cytokines such as TGF-β. Therefore, antagonizing these effects of AT II is a key component of therapeutic strategies to halt progression. Traditionally, the RAS has been considered as an endocrine system. In this view, the glycoprotein angiotensinogen, produced in the liver, is cleaved by renin, which is released from renal juxtaglomerular cells. Thus, AT I is generated, which in turn is converted by the high ACE activity of the lungs into the active substance AT II. AT II is

produced by ACE and also can be formed by other enzyme such as chymase. ACE inhibitors do not inhibit chymase activity.

Blockade of the RAS is based on the different mechanisms of actions of the two drug classes. ACE inhibitor decreased the degradation of bradykinin, a powerful vasodilator in addition to decreased AT II formation. The beneficial effect of ACE inhibition is independent of the decrease in blood pressure. However, an insufficient response to ACE inhibitor might be explained by incomplete blockade of the ACE enzyme or by the generation of ACE-independent AT II by such as chymase. The incomplete blockade possibly explains the observation that plasma AT II levels return to normal after chronic ACE inhibitor treatment[8].

Treatment with AT_1 receptor blocker may result in more complete blockade of the unfavorable actions of AT II mediated through the AT II type 1 receptor. AT1 receptor blocker does not reduce, but actually increases, the concentration of AT II. This excess of AT II will then bind to and activate AT_2 receptors. In the past it had been assumed that activation of AT_2 receptors is exclusively beneficial[33]. However, evidences have been accumulated which clearly suggest that AT_2 receptors may mediate adverse effects. The activated AT_2 receptor stimulates proinflammatory pathways by induction of NF-κB[37]. Further evidence for potentially adverse effects of AT_2 receptor stimulation is provided by in vivo studies demonstrating that AT_2 receptor blockers exhibit anti-inflammatory effects in models of renal injury and are associated with attenuation of renal damage[37]. Standard AT1RAs do not antagonize the type 2 receptor. Treatment with an ACE inhibitor or an AT_1RA has strikingly different effects on the activation of the AT_2 receptor. ACE inhibition leads, at least initially, to a decrease in AT II formation so that less AT II binds to AT_1 as well as AT_2 receptors. Therefore, treatment with both ACE inhibitors and AT_1RAs may offer synergistic blockade of the RAS, not obtainable with either drug alone.

ACE inhibitors and AT1RAs are now the golden standard of therapies against proteinuric renal disease. Renoprotective effects of these reagents had been attributed to amelioration of intraglomerular hypertension. Recent studies have demonstrated that inhibition of RAS improves the molecular mechanisms to retain glomerular permeability and reduces the amount of proteinuria. ACE inhibitors and AT1RAs induce redistribution of the molecules in the slit diaphragm[38], and improve selectivity of proteinuria in patients with glomerular diseases[39, 40]. Large scale clinical trials confirmed the benefits of ACE inhibitors against a variety of renal diseases with their antiproteinuric effects[41, 42]. Furthermore, recent studies demonstrated that the greater beneficial effect of ACE inhibitors in renal disease patients with higher baseline proteinuria could be explained by the greater antiproteinuric effects in these patients[43]. Thus, one of the protective mechanisms of ACE inhibitors and AT1RAs is the reduction of the amount of proteinuria.

Dual blockade of the renin angiotensin system - Promising treatment for renal protection?

Several major clinical trials have clearly shown that ACE inhibitor treatment slows the progression of renal diseases, including in diabetic nephropathy and also halt the progression of renal interstitial injury in animal experiment. Well-controlled studies have demonstrated that this effect is in part independent of BP control. More recently, with AT_1 receptor antagonists a similarly protective effect on renal function was seen in patients with type 2 diabetes and animal studies using UUO rats. However, neither ACE inhibitor nor AT_1 receptor blockade completely abrogate progression of renal disease. A recently introduced novel therapeutic approach is combination treatment comprising both ACE inhibitor and AT_1 receptor antagonists[44]. Theoretically dual blockade of the RAS with ACE inhibitor and AT_1RAs may provide renal benefit beyond therapy with either drug alone.

Clinical studies with ACE inhibitor and AT_1RA

showed that BP and proteinuria were reduced without hyperkalemia. Because these studies demonstrate a reduction in BP with combination therapy, it is not possible to attribute the reduction in proteinuria to dual blockade alone. In patients with IgA nephropathy, the combination of losartan and enalapril decreased urinary protein excretion, whereas doubling the dose of either medication had no further effect on proteinuria[45]. The combination therapy in patients with nondiabetic kidney disease reduced proteinuria by 59% as compared with 45% in those treated with the AT_1RA alone[46]. However, Agarwal et al[47] showed that combination therapy was not superior to maximal-dose ACE inhibitor therapy in decreasing proteinuria in patients with renal disease. This raises the question of whether combination therapy is superior to maximal dose monotherapy[48]. Although most studies suggest that dual blockade has renal benefit, maximal ACE inhibition has not been used in all studies[47, 48]. These studies also have several limitations to draw firm conclusion about combination therapy in renal disease, such as small sample size, use of surrogate markers of renal protection, and short-term follow-up. Some authors suggested following recommendation; 1) titration of the single agent to maximal dose to control BP and proteinuria, 2) if proteinuria remains greater than 1 g/d, add a second agent to block the RAS further[48].

Animal experiment with UUO showed slightly disappointing outcome after relief of UUO[49]. With ACE inhibitor (enalapril) or AT1RA (losartan) the decrease in the interstitial expansion was nearly 30%, significantly faster than with release alone (14% reduction). Unfortunately, complete recovery of interstitial damage developed after UUO could not be reached even after the treatment of ACE inhibitor plus AT_1RAs. Combination therapy reduced only 20% in the interstitial expansion and was not superior to ACE inhibitor or AT_1RA alone.

Conclusion

Interstitial fibrosis can be reversed by pancreas transplantation for diabetic nephropathy and by early relief of ureteral obstruction for obstructive uropathy to a certain extent. Reversal of fibrosis does not always mean complete recovery of renal function. Release of UUO with/without AT inhibition could not reverse the interstitial damage to the preobstruction level. Partial reversal of interstitial expansion was accompanied by persistently abnormal expression of profibrotic factors and continuous activation of the RAS, which may explain the additional beneficial effect of ACE inhibitor or AT_1 receptor blocker in ameliorating interstitial fibrosis, after the release of ureteral obstruction. However, these treatments did not cause regression of renal damage, suggesting that novel approaches are needed.

A recent experiment showed that CTGF might be included as a potential interesting candidate for antifibrotic treatments[33]. TGF-β is an important regulator of fibrosis, however therapeutic strategies blocking TGF β actions have not afforded the expected beneficial effects probably because of its anti-inflammatory properties. Some experimental data[37] also suggest that AT_2 receptors are involved in the inflammatory cell recruitment in the kidney and the blockade of AT II generation by an ACE inhibitor or by combined blockade of both AT1 and AT_2 receptors, as well as by the inhibition of the NF κB pathway, is necessary to stop the inflammatory process fully in animal experiment.

Combination therapy with ACE inhibitor and AT_1RA or combined blockage of both AT_1 and AT_2 receptor appears to provide further renal protection, but further clinical trials and experimental data are required to determine if combination therapy is superior.

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