

Angiotensin in Progressive Renal Diseases: Theory and Practice¹

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ABSTRACT

Experimental studies indicate that AngII is involved in the process of tissue destruction in chronic renal diseases. This notion has been verified in a number of small- to large-scale clinical studies using angiotensin (ANG) I converting enzyme inhibitors (ACEI). Although the repertoire of the pathophysiologic cascade underlying the progressive destruction of renal tissue has continued to expand over recent years, from proteinuria and physical forces to growth factors and metalloproteinase dysregulations, studies now suggest that AngII is involved in many, if not all, of these processes. Because these expanded pathophysiologic potentials of AngII are based primarily on observations *in vitro*, their significance *in vivo*, and in humans in particular, needs to be established. Recent studies in animals and humans indicate that the role of AngII in renal tissue destruction is subject to the modulation of multiple environmental and genetic factors, such as dietary habit and ACE genotype. Further delineation of the role of AngII in this respect for specific renal diseases and patients will enable us to design an efficient therapeutic intervention for this otherwise most complex problem of today's nephrology.

Key Words: *Glomerulosclerosis, interstitial fibrosis, polymorphism, extracellular matrix, TGF- β*

Studies conducted in various parts of the world have shown that angiotensin (Ang) I-converting enzyme inhibitors (ACEi) are effective in curbing the progressive loss of renal function that often occurs in diabetic nephropathy (1,2). A few studies have also indicated that ACEi are equally effective in the treatment of immunoglobulin A (IgA) nephropathy (3,4). Given the highly limited therapeutic options available today for patients with renal disease who are losing

kidney function, this bright news renewed the research interest in Ang. The nature of research has been remarkably diverse, ranging from classic physiology and immunology to cell biology and molecular genetics, using the whole-kidney clearance method to DNA recombinant technology. Studies have shown that, although the kidney is made up of highly heterogeneous cell populations, many of them possess Ang receptors and can thus become the direct target of Ang actions in disease.¹ This article will review recent research findings, including those that are still in preliminary formats, and will also discuss the clinical significance of these findings.

PROPOSED ROLE OF ANGIOTENSIN

The proposed role of Ang today in the progression of renal diseases is illustrated in Figure 1. It should be noted that each of the contributory mechanisms depicted in the figure is not mutually exclusive, but instead interacts with the others.

For a given factor to be considered a cause of a certain disease or pathological condition, at least three conditions need to be fulfilled, namely that (1) the factor is at an abnormal level or is dysregulated, (2) elimination or normalization of the factor ameliorates the disease, and (3) enhancement in the factor accelerates the disease. In this regard, experimental attempts have heretofore failed to provide the first requirement for Ang to be causal to the progression of renal disease. Thus, in the remnant kidney model, the circulating levels of renin and AngII are below normal control levels. This paradox, as discussed by Rosenberg *et al.* (13), may be resolved when one considers that renin content per residual nephron is elevated or that only the local distribution of components of the renin-angiotensin system (RAS) is altered. Considering the involvement of other, RAS-independent, pathophysiologic mechanisms in the disease progres-

¹ Of the two established Ang receptors, the Ang Type 1 receptor (AT1) mediates virtually all of the known functions of Ang. The renal cells or tissues carrying AT1 include: glomerular mesangial and endothelial cells, juxtaglomerular cells, arterial and arteriolar smooth muscles, vasa recta, proximal convoluted and straight tubules, distal convoluted tubules, thick ascending limb of Henle, and collecting ducts (5-9). These receptor localizations were demonstrated in rodents at the messenger RNA or the protein level by localization of markers on tissue specimens or as bands on gel electrophoresis. Admittedly, however, such visual demonstration of mRNA or protein (or lack thereof) does not necessarily prove the presence (or absence) of functionally significant receptors. Indeed, the AT1 in peripheral vascular smooth muscle cells, where Ang II exerts its potent constrictor actions, is extremely difficult to demonstrate visually. Although Ang Type 2 receptor (AT2) appears in the fetal kidney (9,10), and has been postulated to play a role in the apoptotic events occurring during ontogeny (9), it is hardly demonstrable as a discrete signal in mature kidneys. However, pharmacological inhibition of AT2 has recently been demonstrated to cause natriuretics (11). Although AT2 does mediate an apoptotic event in other tissues (12), a significant role of AT2 in renal diseases has not been demonstrated.

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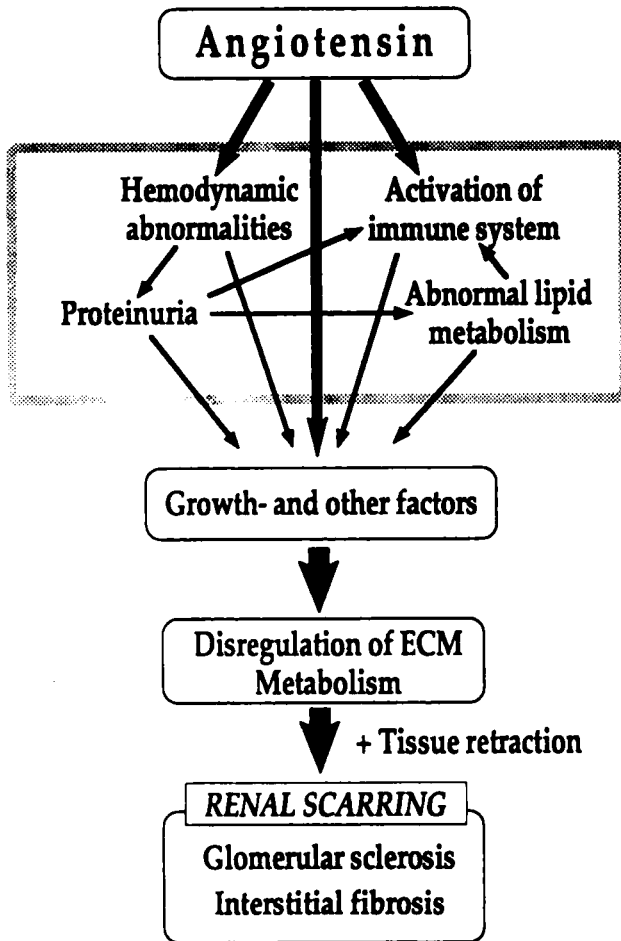


Figure 1. Suggested role of angiotensin in the progression of renal diseases. Currently, two major processes are considered central to renal scarring, i.e., glomerular sclerosis and interstitial fibrosis. Expansion of extracellular matrix (ECM) and tissue retraction are believed to underlie both these processes. Although the role of angiotensin in tissue retraction has heretofore been poorly defined, angiotensin has been shown *in vivo* and *in vitro* to modulate both synthetic and degradation processes of ECM through the modulation of growth factors, cytokines, and other factors. Although angiotensin can directly modulate these factors, it is also capable of affecting two key events that are linked to modulation of these factors, namely, hemodynamics and the immune system. A variety of studies have been performed in recent years to establish the linkage between these key events, as discussed in the text.

sion, the “normal” or even “below-normal” RAS activities in chronic renal diseases may be considered inappropriately high for the volume status and vasomotor tone at that time. This notion is reminiscent of the discovery of the significant roles of RAS in so-called low-renin hypertension. In low-renin hypertension, although renin levels are low in absolute terms, the renin activity is considered to be inappropriately high for the prevailing volume status and vasomotor

tone. This notion has indeed been attested to by the demonstration that ACEi are often highly efficacious in controlling blood pressure in this condition.

Glomerular Hemodynamics

Studies using ACEi in animal models of chronic renal failure demonstrated that ACEi protect glomeruli from progressive sclerosis, often in degrees far greater than one would expect considering their effect on systemic blood pressure (14–18). Unlike capillary beds in other organs, the glomerulus is linked in a series with a postcapillary sphincter, i.e., the efferent arteriole. This allows the glomerular capillary pressure to be regulated independently of the systemic blood pressure. Moreover, ACEi, in contrast to other vasodilators, lead to preferential dilation of this postcapillary sphincter in some experimental settings and cause a profound fall in glomerular pressure (14–18). These findings led to a hypothesis that the abnormally high glomerular pressure causes the progression of glomerular sclerosis, and that the unique effectiveness of ACEi to attenuate this progression is attributed to their potent efferent arteriolar dilative action. This hypothesis gained support from *in vitro* experimental findings that mechanical stress on cultured mesangium cells can increase extracellular matrix (ECM) production. Mechanical stretch was shown to activate the Ang cascade within the glomerular cell (19,20). However, in several experimental settings (21–27), ACEi were shown to be effective in attenuating the tissue destruction of nonrenal organ vascular beds, which lack postcapillary sphincters. In fact, for this very reason, ACEi are now preferred antihypertensive agents for patients with hypertensive or ischemic cardiomyopathy. Although these observations establish the existence of a hemodynamic-independent salutary effect of ACEi, the possibility still remains that the reduction in blood pressure, whether systemic or local, that is achieved by ACEi may also contribute to their structure-preserving effect (28).

Hypertension emerged as the most universal risk factor known for a poor prognosis in many forms of glomerulopathy, including IgA nephropathy and diabetic nephropathy (29,30). Conversely, among a variety of morphological entities of glomerulopathies, glomerular sclerotic change is most frequently associated with hypertension (31). The classic debate, therefore, continues to be whether the systemic hypertension causes, or results from, glomerular damage (32).

Mesangial Macromolecular Processing

On the basis of the localization of exogenously injected ferritin in the subendothelial space and cytosolic compartment of mesangial cells, Farquhar and Palade (33) proposed phagocytic functions by mesangial cells in the removal of “filtration residue” from the circulation. These phagocytic mesangial cells are believed to function as a local reticuloendothelial system and to play a major role in the deposition or removal of

immune complex. Colloidal gold particles (10 nm in diameter) coated with fresh serum can be endocytosed by cultured mesangial cells and delivered to their lysosomes (34). Of particular interest, preincubation of mesangial cells with AngII resulted in enhanced uptake of gold particles coated with IgG_{2b} (35). These findings echo previous observations *in vivo* that AngII given exogenously increases mesangial uptake and delays clearance from the mesangium of tracer macromolecules in normal rats (36–38). These findings are also reminiscent of earlier findings that AngII increases the phagocytic activity of macrophages (39–41). Evidence supporting the notion that the promotion of mesangial phagocytic action by AngII may have pathophysiologic importance exists in studies in the animal model of puromycin aminonucleoside (PAN)-induced nephrosis. Thus, although mesangial macromolecular uptake was increased in PAN rats (42), the enhanced mesangial uptake of radiolabeled, heat-aggregated IgG in PAN rats was nullified by an acute infusion of saralasin (300 µg/kg per min) (43). PAN rats, which are characterized by glomerular morphologic changes indistinguishable from human minimal change disease in the early stage, develop mesangial lesions typically seen in the late stages of focal glomerular sclerosis (44). Moreover, administration of an ACEI markedly attenuates the progression of glomerular lesion to focal glomerular sclerosis (45), a finding supporting the possibility that enhanced macromolecular deposition and phagocytosis by mesangial cells, under the action of AngII, form a crucial intermediary step leading to glomerular sclerosis (46,47).

Proteinuria

Renin or AngII administration enhances urinary protein excretion (48–50). It is now well established that this proteinuria involves increased passage of protein through the glomerular capillary wall (49). Although such an increase in macromolecular traffic across the capillary wall is attributed primarily to altered permeability properties of the glomerular basement membrane with relation to macromolecules, other factors extrinsic to the capillary wall—specifically, hemodynamic factors—may also contribute (51).

A recent series of animal experiments added a new dimension to our understanding of the kinetics involved in Ang-induced proteinuria, identifying the role for *endogenous* Ang. In a rat model of progressive renal failure, marked proteinuria was present, along with an abnormally high glomerular capillary pressure (52). It was evident that the proteinuria accompanied an increase in fractional clearances of neutral dextrans for large dextrans ($\geq 44\text{\AA}$). An acute infusion of a large dose of a calcium channel blocker can largely and promptly near-normalize these indices, including the glomerular capillary pressure and sieving defect. The glomerular sieving defect in the rat model is primarily attributed to an abnormal increase

in the number of large nonselective pores on the glomerular capillary wall, which in turn is caused by a marked elevation in glomerular capillary pressure. This notion has been supported in other animal studies (53) and in a clinical study as well (54). This provides the attractive explanation that ACEI-induced reductions in proteinuria in patients may, in part, be secondary to reductions in the number of large nonselective pores that occur concomitantly with reductions in glomerular capillary pressure.²

Because proteinuria precedes the progressive destruction of renal architecture in virtually all kidney diseases, it is quite normal that proteinuria has been alleged to be causally linked to the damage of the kidney. In this regard, arguably one of the most relevant findings is the one made by Gansevoort *et al.* (56) that, among a nondiabetic patient population given ACEI, a strong correlation exists between the magnitude of suppression of proteinuria achieved shortly after initiation of ACEI *versus* the degree of attenuation of progressive loss of GFR that followed thereafter.

Nevertheless, experimental data directly supporting the notion that proteinuria indeed causes renal damage is lacking, as the manipulation of proteinuria alone is practically impossible in humans or animals.³ Assuming that proteinuria does indeed cause renal damage, what type of pathophysiologic cascade would be operating? The hypothetical mechanisms proposed thus far are depicted in Figure 2 (reviewed in 74,75). ACEI have been shown to reduce the plasma lipid level in animal models (76,77). The renal protective effect of ACE inhibition on patients with massive proteinuria may, therefore, be mediated in part by its lipid-lowering capacity (Figure 1).

Extracellular Matrix Synthesis

Accumulation of ECM in the mesangial region and collapse of the glomerular capillary wall are the hall-

² Improvement in size selectivity of the glomerular capillary wall after administration of ACEI for a few weeks was documented in patients with IgA nephropathy (56). In such a relatively chronic study, this improvement likely reflects both the acute hemodynamic-dependent changes in the wall properly discussed above and a salutary effect of ACEI on the glomerular structure which takes place over the period of weeks or months.

³ It has also not been resolved which specific component of protein, if any, is responsible. In this regard, the congenital analbuminemic rat strain of Nagase is an interesting model to examine. When kidney disease was induced by adriamycin injection or subtotal nephrectomy in these rats, they showed less proteinuria than normal control rats, as expected. However, these analbuminemic rats developed the same degree of sclerosis as Sprague-Dawley rats (57,58), suggesting that urinary albumin alone does not play a major role in the progression of renal sclerosis. In contrast, when rats were repeatedly infused with BSA, they developed proteinuria, glomerular sclerosis, and interstitial inflammatory changes (59). Although circulating antibodies to BSA were not detected in this protein-overload model (BSA is a poor immunogen in rats), the possibility was raised that the lesions may have resulted from an activation of cellular immunity or from some nephrotoxicity specific to BSA. It thus appears essential to ascertain the effect of homologous protein loading. There are only two studies that addressed this issue. In one study, injection of rat albumin resulted in no microscopic changes in the glomerulus or the interstitium (60). In another, two uninephrectomized rats were injected with rat albumin and developed focal tubulointerstitial nephritis, although the disease was less severe than in those rats whose disease was induced by BSA (59). Thus, the evidence for the pathogenic role of albumin in the urinary protein is at best conflicting, whereas the possibility remains that nonalbumin components of proteinuria have a greater pathogenic significance.

marks of glomerulosclerosis. Mesangial cells, and perhaps epithelial cells as well, serve as the source of mesangial matrix. Major components of the mesangial matrix in normal kidneys include the Types IV and V collagens, laminin, fibronectin, entactin, heparan sulfate proteoglycan, and chondroitin sulfate proteoglycan (reviewed in 78,79). Under normal conditions, the balance between the production and degradation of these components is maintained, whereas in glomerulosclerosis, the balance is disrupted and both the quantity and quality of ECM change. Experimental animal models for glomerulosclerosis are characterized not only by overexpressions of the matrix components that are normally expressed (Type IV collagen, laminin, heparan sulfate proteoglycan) but also by abnormal *de novo* synthesis of matrix molecules that are not normally expressed (Types I and III collagen) (80).

Studies have shown that AngII has the capacity *in vitro* (i.e., without involvement of an alteration in systemic milieu, whether hemodynamic or humoral) to induce cell proliferation, hypertrophy, and expression of immediate early genes (such as *c-fos*) and growth factors (including transforming growth factor β [TGF- β] (Figure 3) and platelet-derived growth factor [PDGF]) in cardiomyocytes, vascular smooth muscle cells, glomerular mesangial cells, and renal proximal tubule cells (81,101–107). Moreover, *in vitro* studies have shown that AngII is capable of affecting the production and degradation of ECM (108). Besides, ACEi administration leads to a downregulation of the ECM expression in kidney disease models. Thus, in diabetic rats, enalapril attenuates the otherwise abnormally upregulated mRNA for α 1(I), α 1(III), and α 1(IV) collagen, and laminin B1 and B2 (109). The first unequivocal *in vivo* evidence that local activation of RAS can induce ECM expansion in glomeruli was provided by Arai *et al.* (110). *In vivo* transfection of the renin and Ang genes with liposomes induced ECM expansion and Types I and III collagen expression in glomeruli, whereas there was no change in the contralateral kidney. This short-term study, however, did not demonstrate development of sclerotic lesions.

Administration of a small dose of Ang Type 1 (AT1) receptor antagonist (a dose insufficient to affect systemic blood pressure) remarkably protected the kidney from nephrosclerosis in stroke-prone spontaneously hypertensive rats and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (111,112), although the effect of an ACEi in the latter model was unremarkable in other reports (113). The elevation of the mRNA of TGF- β , Types I, III, and IV collagens, and fibronectin (characterizing untreated controls) were remarkably attenuated, suggesting that the enhanced gene expression of renal TGF- β and ECM components in these rats is mediated by a blood pressure-independent action of AngII through AT1. Several *in vitro* experiments showed that AngII can stimulate the production of ECM component in mesangial cells. AngII stimulates the synthesis of mRNA and protein in Type I collagen, fibronectin, and biglycan in mesangial

cells via the AT1 receptor (81,104–106,114). Further, Kagami *et al.* showed that TGF- β is induced before the increase of ECM proteins in response to AngII, and that a neutralizing antibody to TGF- β remarkably reduces the induction of ECM proteins by AngII (106). Another *in vitro* study has also shown that anti-TGF- β antibody attenuates the AngII-induced upregulation of fibronectin synthesis by approximately 60% (114). Therefore, the increase of ECM protein by the AngII stimulation in some settings appears to be mediated primarily through TGF- β (Figure 3). One study reported that AngII stimulates not only the production of TGF- β mRNA but also the conversion of latent TGF- β to an active form (114).

The intermediary role of other growth factors or the existence of direct AngII action in the AngII-mediated ECM expansion remains unknown. Local overexpression of PDGF-B in the kidney can induce mild mesangial expansion in addition to mesangial cell proliferation (115). In vascular smooth muscle cells, AngII induces PDGF-A (116). These properties suggest a possible role of PDGF in the AngII-induced ECM increase. Indeed, prevention of the development of glomerular sclerosis by using ACEi in an animal model was shown to accompany a downregulation of local PDGF gene expression (117). AngII also induces interleukin-6 (IL-6) in mesangial cells (118), and IL-6 transgenic mice in which IL-6 is massively overproduced developed marked expansion of ECM (119). However, because AngII itself is a relatively weak inducer for IL-6, it remains unknown whether AngII can cause ECM expansion through this mechanism to a significant extent.

The vascular endothelial cells play a critical role in many forms of vascular disease. Experimentally, vascular endothelial cell injury results in local upregulation of TGF- β and AngII. Lee *et al.* have shown that, in the rat remnant kidney, angiotensinogen mRNA, as well as TGF- β 1, fibronectin and laminin B1 transcripts, are abnormally expressed in endothelial cells, particularly in the dilated capillaries, of the glomerulus before the development of sclerosis (120). The AT1 receptor antagonist (AT1RA) inhibited these expressions. Thus, local upregulation of AngII in the injured endothelium may trigger a cascade of TGF- β and ECM protein synthesis. A recent preliminary study has shown *in vitro* that a relatively subtle (i.e., far below that required for mesangial cells) mechanical stretch can enhance the ACE gene expression in cultured glomerular endothelial cells (20).

In the earliest stages of glomerulosclerosis, an injury to glomerular epithelial cells can be demonstrated. However, little is known about the effect of AngII on epithelial cells. It has been reported that TGF- β acts on glomerular epithelial cells and activates the production of Type IV collagen (121), suggesting that ECM production by epithelial cells can be indirectly activated by AngII via TGF- β secreted in a paracrine mode.

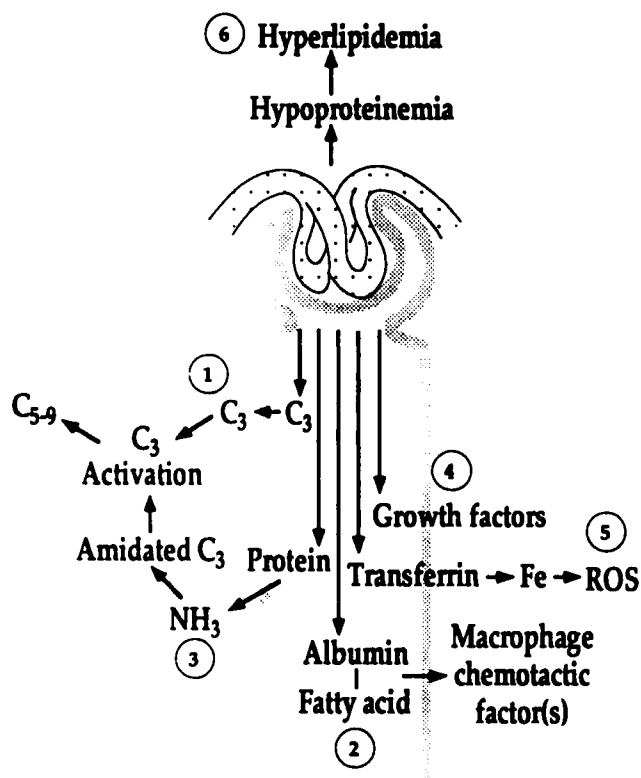


Figure 2. Suggested pathophysiologic role of proteinuria. (1) In protein overload nephritis, deposits of complement component 3 (C3) and the membrane attack complex of complement (C5b-C9) are frequently observed along the luminal border of tubular epithelial cells (59), and are capable of activating complements via the alternative pathway (61). Thus, it is conceivable that C3 permeates the glomerular basement membrane (GBM) and becomes activated by tubular cells. The activated C3, in turn, may trigger the cascade reaction of complements, resulting in the generation of C5b-C9, which may induce tubular cell injury. (2) In BSA overload nephritis, the urine was shown to contain a macrophage chemotactic factor, which is a nonpolar lipid, is acid- and heat-sensitive, alkaline-resistant, and distinct from PAF, leukotrienes, or thromboxane A₂ (62). BSA induced the same macrophage chemotactic factor in cultured proximal tubule cells. BSA is known *in vivo* to bind fatty acid. When the lipid was removed experimentally from the BSA, the BSA no longer induced macrophage chemotactic factor(s) in tubular cells. These results suggest that fatty acid-bearing albumin is metabolized into macrophage chemotactic factor(s) in proximal tubules, thereby triggering monocytes/macrophage infiltration. (3) Increased tubular protein load is thought to activate protein catabolism in tubular cells, which results in increased ammonia production. In fact, it has been shown that urinary ammonia production is highly correlated with proteinuria (63). High concentration of ammonia induces amidated C3, which promotes formation of C5b-C9 and activates release of reactive oxygen species from leukocytes. (4) Growth-stimulatory substance(s) pass through GBM and act on tubular cells. Bruton *et al.* recently showed that serum, but not albumin nor transferrin, applied to the apical surface of

The speculated molecular mechanism of the AngII-TGF- β -ECM axis is illustrated in Figure 3.

Extracellular Matrix Degradation

ECM can be accumulated not only by upregulation of its synthesis but also by downregulation of its degradation. The importance of this degradation mechanism for the development of glomerulosclerosis is attested by experimental demonstrations that, when accumulation of ECM was markedly attenuated, established glomerulosclerosis can be reversed (122,123). ECM proteins are degraded by a number of proteinases. The latter are classified into three categories: serine proteinases, matrix metalloproteinases (MMP), and cysteine proteinases. Among these, serine proteinases and MMP play major roles. Serine proteinases include plasmin, leukocyte elastase, and cathepsin G. MMP include (1) interstitial collagenases, such as MMP-1 and MMP-8, (2) Type IV collagenases, such as MMP-2 and MMP-9, and (3) stromelysins. Each member of the MMP has its own substrate specificity. MMP are secreted in inactive forms, and the conversion to active enzymes requires plasmin, which also plays a central role in fibrinolysis. Plasmin is generated from plasminogen by the action of plasminogen activators (PA), which are also serine proteinases. PA are also activated by plasmin. Thus, the PA-plasmin

tubular cells stimulated production of PDGF and fibronectin. Further characterization of this factor showed that the molecular weight was 100 to 140 kd, suggesting that this factor can be filtered in glomerular disease (64). (5) Separately, a study on iron-deficient rats (165) led to a suggestion that iron dissociated from transferrin in acid tubular fluid may catalyze free radical formation, which, in turn, causes tubulointerstitial injury. (6) Besides the possible direct effects on the tubulointerstitium discussed above, proteinuria, when it causes hypoproteinemia, can affect the progression of renal disease in an indirect fashion by affecting lipid metabolism. The lipid abnormality in nephrotic syndrome includes increased cholesterol, triglyceride, phospholipid low-density lipoproteins (LDL), very-low-density lipoproteins, high-density lipoprotein 3, lipoprotein(a), apoproteins B, CII, and E, and decreased high-density lipoprotein 2 (reviewed in 66). The mechanism of hyperlipidemia in nephrotic syndrome is multifactorial. Both an increased rate of lipoprotein synthesis and a defective lipoprotein catabolism are present. Such abnormalities in lipid metabolism can accelerate glomerular and tubulointerstitial injury, because administration of lipid-lowering drugs and a low lipid-containing diet attenuate renal injury in experimental nephrotic syndrome (reviewed in 67). The mechanism by which hyperlipidemia accelerates renal injury has not been fully elucidated. In animal models of nephrotic syndrome, lipid is deposited both in the mesangium and the tubulointerstitium (68,69). Lipid deposition promotes mononuclear cell infiltration (70). LDL has also been shown to increase the synthesis of ECM proteins in cultured mesangium cells (71). In addition, oxidized LDL is cytotoxic to cultured mesangium cells and stimulates the production of thromboxane A₂, a potent vasoconstrictor (72,73).

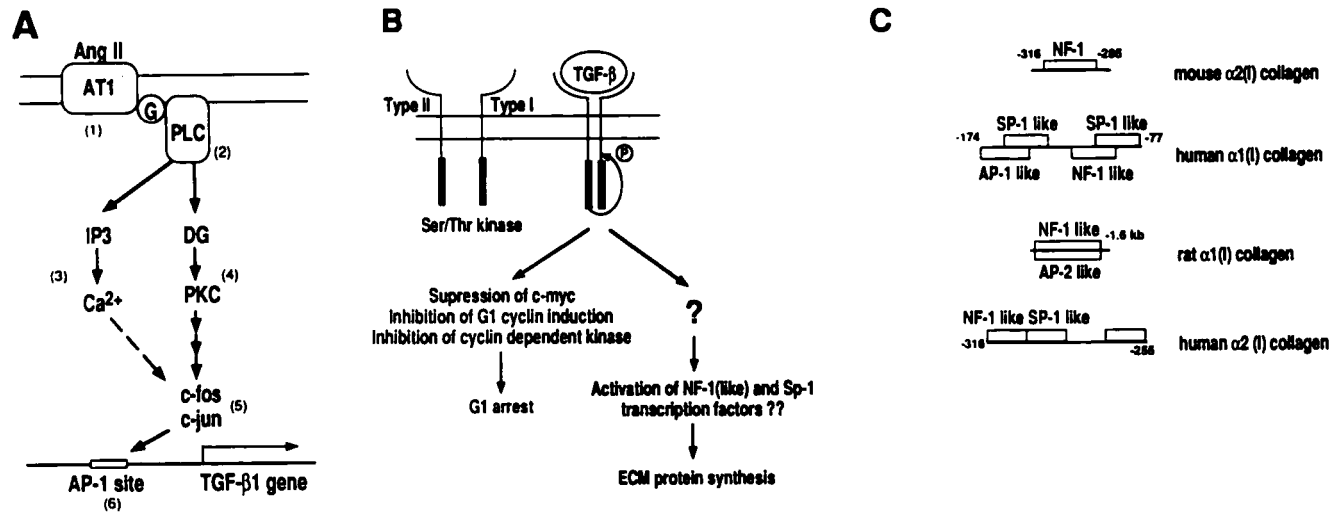


Figure 3. Speculated mechanism of induction of ECM protein production by Ang II. **(A)** TGF- β induction by Ang II. (1) Stimulation of ECM protein synthesis by Ang II is mediated by AT1 receptor (81). (2) AT1 receptor is coupled to phospholipase C, which generates inositol triphosphate (IP3) and diacylglycerol (DG). (3) IP3 activates the release of Ca²⁺ from intracellular stores to cytoplasm. (4) Ca²⁺ and DG synergistically activates the protein kinase C (PKC). Increase in intracellular Ca²⁺ was shown to be sufficient to cause the activation of MAP kinase in cardiac myocytes (82). In vascular smooth muscle cells and cardiac myocytes, induction of TGF- β by Ang II is PKC-dependent (83). Signal of PKC is thought to be mediated by transcription factor, AP-1, which is composed of c-fos and c-jun. (5) Ang II induces c-fos and c-jun in cardiac myocytes, fibroblasts, and vascular smooth muscle cells (reviewed in 84). It has been shown that the Ang II response element of the c-fos gene is SRE (serum response factor) (85). PKC directly phosphorylates and activates Raf-1 kinase (186). Activated Raf-1 kinase triggers MAP kinase cascade, resulting in phosphorylation of the ternary complex factor, which associates with SRF transcription factor and augments the transcription through SRE. c-jun is induced later than c-fos by the stimulation of Ang II (87). c-jun can be autoinduced by AP-1. The effect of Ang II on another important kinase cascade, JNK(jun kinase) pathway has not been reported. (6) The heterodimer of c-fos and c-jun can bind to AP-1 site and activate transcription. In the promoter region of the human TGF- β gene, there are AP-1 binding sites, which are responsible for the induction by several oncogenes including jun and fos (88). It has not been confirmed whether the Ang II actually activates the TGF- β gene via the AP-1 binding sites, however. This simple scheme evokes a question: Do all of the factors that activate the PKC cause the same reaction if cells have the receptor? The signal transduction system may not be so simple. In hepatocytes, a tyrosine kinase inhibitor was shown to block the c-fos expression by Ang II (89). In addition, in myoblasts, reactive oxygen species mediate the AP-1 induction by Ang II (90). **(B)** Activation of ECM protein production by TGF- β . (1) The important receptors that are involved in the signal transduction of TGF- β are Type I and Type II receptors, which are unique in that they have serine threonine protein kinase activity, unlike other growth factors or cytokines, whose receptors have (or are coupled with) protein tyrosine kinase activity. (2) After ligand binding, Type I and Type II receptors form a heteromeric complex, and the Type II receptor kinase (which is constitutively active) then phosphorylates the Type I receptor, thereby activating the Type I receptor kinase (91). (3) Although some molecules associated with the receptors have been identified (FKBP, TRIP-1) (92,93), their specific functional roles are unknown. Most studies of the TGF- β signal transduction have focused on its effect on cell cycle regulation (reviewed in 94). The signal transduction pathway to ECM production is unknown. A recent report has indicated that a member of the MAPKKK family, TAK1, may mediate this signal (95). **(C)** On the other hand, in the genes for several ECM proteins (including mouse $\alpha 2(I)$, rat $\alpha 1(I)$, human $\alpha 1(I)$, and human $\alpha 2(I)$ collagens), TGF- β responsive elements have been identified (96–99). The TGF- β responsive elements of these four genes contain a CTF/NF-1 (CCAAT box binding transcription factor/nuclear factor 1) binding-like motif. NF-1 is a ubiquitous nuclear protein and its amount does not change by the stimulation of TGF- β . The TGF- β responsive element of rat $\alpha 1(I)$ collagen gene has a motif similar to NF-1 motif, and nuclear protein, although different from NF-1 protein, binds to this sequence. Additionally, the TGF- β response elements of human $\alpha 1(I)$ and $\alpha 2(I)$ collagens contain Sp-1 binding motif. Sp-1 is another ubiquitous nuclear protein. So far, the precise mechanism by which TGF- β activates these transcription factors is not known. A recent study has shown that the TGF- β domain of CTF-1 is located on the proline-rich transactivating domain, which interacts with histone H3, suggesting that TGF- β may regulate transcription by modulating chromatin dynamics (100).

system forms a positive feedback loop. Because a large amount of plasminogen is present in all tissues, production of a small amount of PA results in a large amount of plasmin, leading to activation of MMP (reviewed in 124).

As a regulator of this system, PA inhibitor-1 (PAI-1)

plays a pivotal role. PAI-1 deficiencies are associated with a bleeding tendency in humans, and increased PAI-1 activity is associated with a thromboembolic phenomenon. The importance of PAI-1 in ECM generation in the kidney was shown by a study in which administration of PAI-1-neutralizing antibodies to

cultured mesangium cells resulted in a several-fold increase in the amount of ECM degradation (125). Moreover, normal mouse kidneys contain a very low level of PAI-1, whereas in a mouse model of lupus nephritis, PAI-1 is expressed in endothelial cells, parietal epithelial cells, tubular epithelial cells, and infiltrating mononuclear cells in the tubulointerstitium (126).

Several groups of investigators have shown that AngII increases PAI-1 mRNA in cultured vascular smooth muscle cells, vascular endothelial cells, and astroglial cells (127–130). The AngII-stimulated PAI-1 increase occurs within 2 h and is not affected by cycloheximide (127), suggesting that this induction does not require new synthesis of an intermediate protein. In one report, the PAI-1 induction by AngII in endothelial cells was mediated by both AT1 and AT4 receptors (128), whereas in another report, the induction was mediated solely by the AT1 receptor (129). *In vivo* infusion of AngII in humans resulted in an increase in the circulating level of PAI-1 (131). It was also reported that ACEi administration to humans lowered plasma PAI-1 levels (132). Thus, AngII can attenuate fibrinolytic activity and ECM degradation via the PAI-1–PA-plasmin system.

Tubulointerstitial Changes

In chronic progressive renal diseases, structural damage is commonly observed in the tubulointerstitium, which is particularly fibrous by nature. One study (133) on human biopsy specimens from a variety of diseases indeed demonstrated the presence of an impressive correlation between the histological abnormality of tubulointerstitium and the severity of azotemia. It has been shown that ACEi administration can ameliorate the tubulointerstitial changes in experimental models. Diamond *et al.* showed that an ACEi reduced the severity of interstitial fibrosis, the extent of tubular dilation, and the number of intratubular casts in the chronic progressive phase of aminonucleoside nephrosis (134). Kaneto *et al.* observed that an ACEi significantly blunted the increase of TGF- β 1 mRNA in the tubules of experimentally obstructed rat kidneys, in which a marked interstitial fibrosis would otherwise develop (135). In this model, the structure of glomeruli appears normal, suggesting that AngII activates TGF- β without involvement of the glomerulus.

In vitro experiments by Wolf *et al.* using MCT, a proximal tubular cell line, showed that AngII increases Type IV collagen and TGF- β in these cells and that, through the expression of TGF- β , AngII acts on MCT cells as a hypertrophic factor (107).

In tubulointerstitial nephritis, collagen fibers appear to be produced primarily by interstitial fibroblastic cells. Fibroblastic cells are not uniform, and their phenotype changes with pathologic conditions and with the stimulation of cytokines (136). During wound healing, fibroblastic cells exhibit several features

characteristic of smooth muscle cells, including expression of α -smooth muscle actin. These cells, called myofibroblasts, are believed to play a retractile role in granulation tissues. α -Smooth muscle actin has been demonstrated in tubulointerstitial lesions of various human diseases and experimental models (137). It has not been experimentally established whether the myofibroblast has ECM generation capacity greater than that of ordinary fibroblasts. Notably, interstitial cells from kidneys with interstitial nephritis can produce a larger amount of collagen *in vitro* compared with those from normal kidneys (138). In this context, it is very interesting that *in vivo* infusion of AngII into normal rats dramatically upregulates the α -smooth muscle actin in the interstitium, suggesting that AngII may promote the phenotypic modulation of fibroblasts or expand the specific population of myofibroblasts. Indeed, rats chronically infused with AngII develop tubulointerstitial injury with tubular atrophy and dilation, cast formation, interstitial monocytic infiltration, and interstitial fibrosis with Type IV collagen deposition (139). The injury is associated with an increase of PDGF-B mRNA within tubular and interstitial cells. Furthermore, osteopontin, which has a murine macrophage chemotactic activity *in vivo*, is expressed focally in cortical renal tubules, and monocytes/macrophages subsequently accumulate almost exclusively around the highly expressed osteopontin (140). This finding, together with the fact that AngII induces osteopontin in cultured vascular smooth muscle cells (141), indicates that AngII induces macrophage infiltration via the induction of osteopontin.

Collectively, the above *in vivo* and *in vitro* data suggest that AngII is involved in tubulointerstitial injuries by modulating expression of growth factors, interstitial cell phenotype, and inflammatory cell responses.

Involvement of the Immune System

Angiotensinogen is one of the acute phase proteins whose expressions are augmented by inflammatory cytokines. In granulomatous inflammation, such as that seen in sarcoidosis, the serum ACE level is elevated, and granulomatous macrophages produce ACE. Thus, a functional relationship may exist between the RAS and the immune system. It is believed that a small population of resident macrophages are present within normal glomeruli. In many forms of human glomerulonephritis, there is an accumulation of macrophages within not only the glomerulus but also the interstitium (142). The importance of macrophages in the initiation and the progression of renal injury has been demonstrated in studies in which macrophage deletion by x-irradiation or anti-macrophage serum attenuated glomerular and tubulointerstitial injuries (143,144).

One significant study showed that captopril decreases granuloma size in animal experiments (145). As previously mentioned, AngII infusion induces mac-

rophage infiltration (139). Also, in a rat model of unilateral ureteral obstruction, administration of an ACEi (enalapril) ameliorated interstitial monocyte/macrophage infiltration, which is seen as early as 4 h after ureteral ligation (146,147). The salutary effect of ACEi on atherosclerosis is also relevant to the relationship between the RAS and the macrophage. In the early stages of atherosclerosis, low-density lipoprotein (LDL) accumulates in the subendothelial space of vessel walls and is oxidized by the endothelial and smooth muscle cells, and the macrophages are then recruited into the arterial intima. Oxidized LDL is then taken up by the macrophages via scavenger receptors on the cell surface. In this regard, because of the morphological and functional similarity existing between mesangial cells and vascular smooth muscle cells, as well as the fact that lipid and apoprotein B can be seen within sclerotic glomeruli, glomerulosclerosis is thought to share common pathogenic mechanisms with atherosclerosis (148). ACEi has been shown to prevent atherosclerosis in various animal models without changing the plasma lipid profile, and even without lowering blood pressure (149,150). These findings collectively suggest that inhibition of ACE can directly suppress the accumulation of macrophages in injured tissues.

Three mechanisms have been proposed for this ACEi trait. First, AngII may directly activate macrophages. Macrophages have AngII receptors (151). Some *in vitro* studies showed that AngII acts on macrophages and increases intracellular cAMP production, protein tyrosine phosphorylation, and tumor necrosis factor α production, enhances their phagocytotic activity, and promotes their adhesion to endothelial cells (39-41, 151,152). However, the significance of these events, especially during *in vivo* situations, has not been established. Second, AngII may induce the release of monocyte/macrophage chemotactic factor(s). AngII infusion leads to tubulointerstitial nephritis, in which osteopontin is expressed before macrophage infiltration, suggesting that the effect of AngII on macrophage activation is mediated by osteopontin (140; see above), although no direct evidence for this hypothetical mechanism has been heretofore provided. One study showed that the amino- and carboxyl-terminal tetra peptides of AngII have chemotactic activity for human mononuclear leukocytes, including monocytes (153). Third, the kinin system may be involved. In rats with unilateral ureteral obstruction, enalapril markedly reduced monocyte/macrophage infiltration, whereas an AT1RA, SC-51316, had almost no effect on monocyte/macrophage infiltration (146). It has also been demonstrated that, in cholesterol-fed rabbits, enalapril reduced the atherosclerosis, whereas SC-51316 showed no significant attenuation (150). Thus, the antiatherosclerotic effect of ACEi appears to be mediated not through the suppression of AngII but through an inhibition of kinin degradation. ACEi increases bradykinin, and the increased bradykinin can stimulate the production of

prostacyclin or nitric oxides (NO) in endothelial cells; this in turn can reduce the monocyte adhesion or chemotaxis. The involvement of NO in this cascade has been shown by an experiment using NO synthase inhibitor (154). When an NO synthase inhibitor (N^o-nitro-L-arginine methyl ester [L-NAME]) was administered together with enalapril to rats with unilateral ureteral obstruction, the monocyte/macrophage infiltration was not ameliorated.

Effects of AngII on other types of immune cells have also been reported. Vance and Kelly (155) recently showed that a murine nephritogenic T cell clone, 53.28.1, which can induce interstitial nephritis in naive syngeneic recipients after adoptive transfer, expresses mRNA for both AT1 and AT2 receptors, and that AngII augments proliferation of this T cell clone (under the stimulation by anti-CD3 antibody). They also showed that ACEi and AT1RA (but not AT2 antagonists) inhibit the T cell proliferation in the absence of exogenous AngII. These results suggest that AngII may play a role in clonal expansion of nephritogenic T cells.

Other Potentially Important Mechanisms

The effectiveness of ACEi on atherosclerosis has been studied in another regard, *i.e.*, the pathophysiologic role of lipid modification. Keidor *et al.* (156) reported that the LDL derived from patients with essential hypertension is more susceptible to lipid peroxidation than that from control subjects. The susceptibility was decreased by treatment of patients with ACEi. These authors also showed that LDL can bind AngII and that AngII has a stimulatory effect on copper-mediated oxidation of LDL, as well as on LDL degradation by macrophages. Because glomerulosclerosis is thought to share common pathogenic mechanisms with atherosclerosis (148), the oxidized lipid may contribute to the progression of glomerulosclerosis in a similar fashion.

Greene *et al.* and Hostetter *et al.* (157,158) documented a significant role for aldosterone in the establishment of renal lesions in the rat remnant kidney model. They reported that subtotal nephrectomized rats developed hyperaldosteronism and adrenal hypertrophy, which could be normalized by AT1RA administration, and that infusion of aldosterone into subtotal nephrectomized rats treated with AT1RA caused proteinuria. Hyperaldosteronism may, therefore, be an important determinant for proteinuria in this model.

Unique role of AngII in maturing kidneys. When the kidney is in the process of maturation, Ang plays a unique role in the development of both normal and abnormal renal structure. Recent recombinant DNA studies have demonstrated that selective inactivation of the angiotensinogen gene in mouse embryos led to development of kidneys with marked abnormalities, including, among others, arteriolar hypertrophy, interstitial infiltration and fibrosis, and mesangial ex-

pansion (159,160). Thus, endogenous Ang is salutary and, in fact, indispensable for normal growth of the kidney. Similarly, in a mouse model of congenital glomerulosclerosis, administration of ACE inhibitors or an AT1 receptor antagonist early in life resulted in the acceleration of glomerular and interstitial changes (161). In maturing animals, however, Ang has been shown to exert an injurious effect. In experiments by Chevalier *et al.* (162), whereas ureteral ligation in newborn guinea pigs caused a marked impairment of maturational growth of glomeruli, simultaneous administration of an ACEi was shown to attenuate markedly the glomerular growth-preventive effect of ureteral obstruction. It was further demonstrated that renin content was highly elevated in the ipsilateral kidneys. Thus, in this particular setting of renal injury during maturation of the kidney, Ang appears to serve as a potent growth inhibitory factor.

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS

Genotype

Genetic studies have revealed that virtually all of the genes comprising RAS have several forms of polymorphism, raising the possibility that the activity of the RAS may vary among individuals in accordance with each of their genetic makeups. One such polymorphism is the deletion/insertion polymorphism of the ACE gene. The ACE gene consists of 26 exons and spans 21 kilobases on Chromosome 17. Within Intron 16, a polymorphism consisting of the presence or absence of a 287-base pair fragment exists. Although this deletion polymorphism is associated with elevated serum and cellular ACE levels (163–165), its association with blood pressure levels or ischemic heart disease varies among populations of different genetic and environmental backgrounds (166–171). Interestingly, recent studies indicate that gene variants of other components of RAS also affect the risk of hypertension and several forms of cardiac disease. A point mutation of nucleic acid in the angiotensinogen gene (*AGT*) leads to an amino acid substitution of threonine for methionine at Codon 235 (M235T). This *AGT* M235T polymorphism was associated with plasma concentration of angiotensinogen (172), hypertension (172,173), and coronary heart disease independent of, and synergistically with, ACE deletion/insertion polymorphism (174,175). Finally, a nucleic acid substitution of A to C at Position 1166 in AngII receptor Type 1 receptor (*AT1*) gene (A1166C) was found. It has been reported that the A1166C polymorphism is also associated with hypertension (176), and affected the risk of coronary heart disease synergistically with the ACE gene polymorphism (177).

Recent findings (178,179) indicate that the deletion polymorphism in the ACE gene is associated significantly with the progressive loss of renal function in patients with IgA nephropathy, although the polymorphisms of the other genes described above were found not to affect the progression. Also, although results

are variable (180), studies have indicated that in patients with early insulin-dependent diabetes, the deletion/insertion polymorphism of ACE gene is associated with the development of microalbuminuria or proteinuria, which are considered as prodrome to progressive renal failure (181,182). Moreover, more recent studies have shown that, in insulin-dependent (183) and non-insulin dependent diabetes (184), the ACE DD genotype has not only a significant correlation with the loss of renal function, but also a high prognostic value for the progressive nephropathy. In a study concerning IgA nephropathy (178), ACEi was shown to reduce proteinuria most prominently in patients with the ACE DD genotype, suggesting that the pathophysiological contribution of RAS to the progression may vary among individuals according to the genetic makeup of their RAS system.

Environmental Factors

Dietary protein. It is well known that protein intake acutely elevates GFR and intraglomerular pressure, whereas a low-protein diet is associated with low intraglomerular pressure and GFR (185). The latter can also be achieved by ACEi. In addition, dietary protein restriction, like ACEi, slows the progression of chronic renal failure to variable degrees (186–190). Although the mechanism of the protein-induced hyperfiltration is still unknown (reviewed in 191), it has been shown that dietary protein modestly stimulates the RAS (192). However, because ACEi cannot nullify protein-induced hyperfiltration (193), low protein intake appears to modulate glomerular hemodynamics independent of AngII. In accord with this contention, a low-protein diet and ACEi exert additive effects in decreasing proteinuria both in rats and humans (194,195). An animal experiment with puromycin aminonucleoside nephropathy showed that concurrent ACEi administration and low-protein diet can additively reverse glomerulosclerosis (122).

Dietary salt. A high-salt diet decreases systemic renin levels. In the DOCA-salt hypertension model, systemic renin–AngII levels are suppressed; therefore, AngII blockage in this setting cannot decrease blood pressure. Nevertheless, AngII blockage was shown to attenuate progressive renal injury in this model (112). If this is really the case, then, even in this so called “low renin model,” local AngII with significant activity prevails in the kidney, and this “unmeasurable” AngII plays an important role in causing renal injury.

Stress. Renin release is controlled in part by β -adrenergic catecholamine, and various stresses activate the RAS. The chronic exposure of rats to cold temperatures induces hypertension and cardiac hypertrophy (196). Plasma renin activity and responsiveness to exogenous AngII increases after cold exposure, and losartan prevents the hypertension, suggesting that the RAS plays an important role in this setting (196). Immobilization stress was also shown to increase renin levels (197). The effect of such stress on the progression of renal disease has not been well studied.

Exercise. Exercise is known to increase plasma AngII levels. Proteinuria after exercise is common even in healthy subjects, and is known as post-exercise proteinuria. The effect of ACEi on post-exercise proteinuria is controversial. Some authors have reported that ACEi decreased postexercise proteinuria (198,199), whereas others reported no effect (200,201). Interestingly, some human studies found that the increase in plasma AngII levels can be inhibited by nafamostat (a serine protein inhibitor), but not by ACEi (201,202), indicating an involvement of an alternative ACE-independent pathway for AngII synthesis after exercise (202).

The effect of chronic exercise on the course of renal diseases appears to depend on the nature of renal injury. An animal study reported that exercise worsened immune complex-mediated glomerulonephritis in rabbits as assessed by proteinuria (203). In contrast, in another animal experiment of subtotal nephrectomy, daily training ameliorated glomerulosclerosis (204). The role of AngII in these modulatory processes is not known.

Inflammation. Angiotensinogen is one of the acute phase proteins, and its production is accelerated by inflammatory cytokines. Renin is not the only rate-limiting step for AngII production, but AngII production is also influenced by the availability of angiotensinogen. Therefore, in inflammatory conditions such as infection and injury, AngII increases and may exaggerate renal injury.

PHARMACOLOGICAL INTERVENTION IN HUMANS

The ACEi have been well established in clinical studies as organ-protecting agents in two forms of cardiac tissue injuries; namely, congestive heart failure (CHF) and ischemic heart disease.⁴ By inspecting the literature on their pathophysiology and the pharmacology of ACEi in these settings, one must be impressed by the analogy between these progressive cardiac diseases and the progressive renal failure. Thus, in these cardiac injuries also, growth factors

⁴ Maladaptation of the RAS is believed to be involved in the pathogenesis of congestive heart failure (CHF), as the RAS is not suppressed despite the salt retention that uniformly characterizes CHF. The mortality rate ranges from 50% over 5 yr to a 64% 1-yr mortality rate in patients with Class IV CHF. Traditional treatment with diuretics and inotropic agents have failed to improve long-term survival. By contrast, ACEi have been demonstrated to reduce mortality in this disease. Enalapril reduced 6 and 12-month mortality in patients with advanced CHF who were already under treatment with diuretics, digitalis, and vasodilators (205). Patients with CHF and ejection fractions ≤ 0.35 experienced a 16% reduction in mortality with enalapril, compared with placebo (206). Progression from asymptomatic left ventricular dysfunction to clinically evident CHF was reduced by enalapril (207). Enalapril reduced mortality at two years from 25% to 18% when compared with the combination of isosorbide dinitrate and hydralazine (208). ACEi has also been shown to be of benefit in preventing expansion of scar formation and remodeling after myocardial infarctions, which predispose to left ventricular dysfunction, aneurysm formation, and arrhythmias. When ACEi are introduced in the first 2 days after myocardial infarction (MI) in patients with CHF or asymptomatic left ventricular dysfunction, survival is improved (209–211). ACE originating from endothelial cells has been demonstrated in the marginal zone of tissue repair in MI. Its induction is related to the production of collagen in the repair process (212). The beneficial effect of ACEi is most marked in patients with ejection fractions of under 40% (213). Beneficial effects were seen after short- and long-term treatment after MI. (214) Zofenopril reduced mortality and evidence of CHF 6 wk after infarction. Trandolopril was shown to reduce overall mortality, sudden death and severe CHF in post-MI patients with left ventricular dysfunction treated for 24 to 50 months (215).

and cytokines are involved in the process of cell proliferation, hypertrophy, and tissue scarring, and ACEi are found to be advantageous over other antihypertensive agents because of their blood pressure-independent actions. Compared with these heart tissue injuries, however, studies on the progression of renal diseases are few and small in scale, presumably because the incidence of the latter is less common, and the parameters to be followed are less quantitative or more cumbersome to obtain than the former. Nevertheless, convincing clinical evidence has been accumulated to indicate that, at least in some settings, ACEi are uniquely effective over other antihypertensive agents in attenuating the progressive deterioration of kidney diseases.

Responsiveness

Disease entities. The kidney disease in which ACEi was first shown to be beneficial for the maintenance of renal function was scleroderma renal crisis (216). Although there were no controlled clinical trials, ACEi strikingly improved the survival rate of both of the study's patients *per se* and their kidneys, whereas, before the era of ACEi, no patient survived beyond a few months.

Besides this somewhat special condition, in which small renal arteries are primarily affected and renin Ang is systematically upregulated, other more common forms of kidney injury also gain benefit from ACEi. The renal protective effect of ACEi was most convincingly shown in insulin-dependent diabetes mellitus (IDDM). In a randomized, large scale, long-term, double-blind, controlled trial comparing captopril with placebo, it was shown that the ACEi reduced the risk of a doubling of serum creatinine levels over 3 yr by 50% (1). The long-term advantage of ACEi over conventional antihypertensive drugs in renal protection also has been shown in a middle-scale, prospective study in non-insulin-dependent diabetes mellitus with overt nephropathy (217). A report by Cattran *et al.*, although not a randomized controlled study, showed that ACEi also has a beneficial effect on severe IgA nephropathy independent of its blood pressure-controlling effect (3).

Considering the possible mechanisms of the renal protective effect of ACEi, it appears that the renal protective effect of ACEi is predominantly seen in slowly progressive diseases with glomerulosclerosis and/or interstitial fibrosis. Included in this category are obstructive nephropathy, reflux nephropathy, lupus nephropathy, transplant rejection, tubulointerstitial disease, and renal mass reduction. Because of the relatively low incidence of these renal diseases, however, a large-scale, long-term, controlled study has not been done. For these kidney diseases, there are only several short-term, small-scale, or mixed-disease studies. In this regard, an interesting cross-over study was reported by Praga *et al.* comparing captopril and other drugs (218). These authors fol-

lowed up 46 nondiabetic patients with nephrotic proteinuria for a minimum of 12 months before captopril treatment and for, on average, 2 yr after captopril treatment. Patients whose proteinuria decreased by greater than 45% exhibited a remarkable stabilization of renal function, whereas those whose proteinuria decreased by less than 45% showed continuous deterioration of renal function. As mentioned earlier, Gansevoort *et al.* have shown the existence of a correlation between the antiproteinuric effect of ACEi and the attenuation of reduction in renal function (56). Notably, in the study of Praga *et al.*, patients with renal mass reduction, inactive idiopathic crescentic glomerulonephritis, nephroangiosclerosis, and reflux nephropathy showed striking antiproteinuric response, whereas those with membranous glomerulonephritis and idiopathic focal glomerulosclerosis showed poor antiproteinuric response, and those with IgA nephropathy showed intermediate response. Nephropathy with active ongoing immunological process may, therefore, benefit little from ACEi treatment.

There is no convincing evidence for a superiority of ACEi over calcium channel blockers for renal protection in human disease (219–221, reviewed in 222). Some experimental studies have shown that calcium channel blockers decrease afferent vascular resistance and do not decrease intraglomerular pressure (223). Parallel to this phenomenon, ACEi, but not calcium channel blockers, attenuated the progression of glomerulosclerosis in animals. A long-term prospective study in humans showed no difference between ACEi and calcium channel blockers in their capacities to attenuate the progression of renal insufficiency, although some clinical trials showed a greater antiproteinuric effect of ACEi over calcium channel blockers (219). Animal experiments suggested that calcium channel blockers, although unable to reduce intraglomerular pressure, have a greater antiglomerular hypertrophic effect than ACEi, resulting in a comparable effect of ACEi and calcium channel blockers in reducing glomerular injury (224), although some animal studies indicated that calcium channel blockers are less renoprotective (225) or can even augment renal injury (226).

Disease stages. In animal experiments, Ikoma *et al.* showed that, in a given remnant kidney, ACEi therapy attenuates progression most remarkably in nonsclerotic or early sclerotic glomeruli, whereas ACEi exerts no effect on glomeruli with advanced sclerosis (123). In a human study of IDDM with overt nephropathy (1), a remarkable renal protective effect of ACEi was demonstrated in patients with significant renal insufficiency. Thus, in a large prospective study of IDDM, when the patients are categorized into three groups on the basis of their baseline serum creatinine levels—1.0, 1.5, or 2.0 mg/dL—the reduction in risk of a doubling of the serum creatinine level achieved by ACEi in each group was 17, 55, and 76%, respectively. However, the results also pointed to the importance of early treatment, because the group with low baseline

creatinine concentrations did not show deterioration of serum creatinine levels during the follow-up period. The demonstrated efficacy of ACEi in patients with renal insufficiency is reminiscent of the notion that chronic renal diseases are characterized by the presence of nephrons at diverse stages of injury, from very early to late, whereas the renoprotective effect of ACEi may be accomplished primarily through its effectiveness on glomeruli in the early stages of injury. The unequivocal evidence for the efficiency of ACEi therapy in patients with increased serum creatinine levels in this study offers bright prospects for ACEi treatment in other diseases as well, because in some diseases (particularly in diabetes mellitus), glomerulosclerosis is fairly advanced even with normal serum creatinine levels (227). In some diseases, patients with advanced renal insufficiency can also benefit from ACEi treatment (218).

Individual patients. The activity of the RAS is influenced by various individual patient-dependent factors, including genotype, age, race, and sex. As discussed earlier, the ACE DD, Atg M235T, and AT1 A1166C genotypes are related to high levels of activity of the RAS. African Americans and elderly individuals have a high incidence of low-renin hypertension, which is resistant to ACEi therapy (reviewed in 228). The renoprotective effect of ACEi treatment may be different in these groups of populations as well. A study with IgA nephropathy has shown that patients with the DD genotype of the ACE gene achieved the most prominent reduction in proteinuria with ACEi treatment when compared with those with other genotypes. The role of other individual-dependent factors on the renoprotective effect of ACEi has not been evaluated.

Dosage of ACEi agents. As discussed earlier, studies in animal models demonstrated that ACEi attenuate progressive deterioration of kidney structure independently of their effect on systemic blood pressure (111,112,146). Some studies also indicated that when ACEi is given to animals far in excess of the dose required for blood pressure control, it can reverse established sclerosis in some glomeruli (123). These findings are consistent with the *in vitro* demonstration that Ang modulates the metabolism of collagens in cultured renal cells. Similarly, a few clinical studies have observed that ACEi is protective in the kidney, independent of blood pressure, and even in patients without hypertension (1). Notably, the dose recommended for each of the ACEi available in the market today is based on the results of clinical trials assessing their potency of reducing blood pressure. Given that the renoprotective effect of ACEi is independent of blood pressure, it is therefore conceivable that the dose-response curve for renoprotection may be quite different from that for blood pressure. In humans, however, no study has been systematically performed to determine the optimal dose of ACEi for renoprotective purpose in normotensive patients or to examine if dosages higher than that required for controlling

blood pressure impart additional renoprotection in hypertensive patients. Because the dose-response curve of ACEi in inhibiting aldosterone secretion and causing hyperkalemia may likewise be different from that of their antihypertensive effect in humans, clinical tests of these types would require close and careful monitoring of serum potassium levels.

Different Pharmacological Agents

Various ACEi agents. After the development of captopril and enalapril, a number of ACEi have been developed. Each has a different chemical structure, and therefore has different pharmacokinetics. Because ACE molecule is made up of two catalytic domains that are structurally similar, yet functionally heterogeneous, as suggested by a recent study (244), and because the relative affinity to these two separate domains appears different among ACEi (245), biological activity may vary among different ACEi. However, no qualitative difference between these agents has been reported for human use. Some ACEi have additional pharmacological actions, such as β -blocker activities (229) and neutral endopeptidase (ANP degradation enzyme) inhibitory activities (230). The value of such a dual-inhibitor in renal protection has not been evaluated, but inhibition of neutral endopeptidase may be beneficial because an inhibition of neutral endopeptidase results in increase of ANP, thereby facilitating sodium excretion.

Captopril and zofenopril have a sulphhydryl group in their molecular structures. This structural arrangement is thought to be responsible for some of their side effects, such as taste disturbance and skin rash. On the other hand, they exert free-radical scavenger activities. The idea that clinical doses of captopril have a significant antioxidant activity is controversial, although a report showed that captopril, but not enalapril, reduced breath pentane, a product of lipid peroxidation, in patients with chronic heart failure (231). The therapeutic significance of the antioxidant activity of captopril was shown in some experimental models, including ischemia-reperfusion injury of the heart and viral myocarditis (232,233). However, the clinical importance of this possible antioxidant activity has not been recognized.

ACEi versus AT1RA. A new class of nonpeptide Ang inhibitor, AT1RA, has recently become available as an oral antihypertensive agent. The mode of action of these agents is distinctively different from those of ACEi, as the former acts as an AT1RA. Because of this difference, the effectiveness of AT1RA on the progression of renal diseases may also be different. ACEi also acts on the kinin system, and accumulation of bradykinin is thought to cause some of the side effects of ACEi, such as cough and angioneurotic edema. Increased levels of bradykinin have been shown experimentally to dilate efferent arterioles profoundly, reduce proteinuria markedly (234), and prevent macrophage infiltration by increasing NO synthesis

(154). It has recently been demonstrated that, in humans but not in rodents, AngII is produced by nonrenin alternative serine kinase (235). Therefore, ACEi cannot completely block the action of AngII, whereas AT1RA can completely block the AT1-dependent action of AngII. Notably, it is now known that AT1RA also exerts nonspecific actions. Thus, it has been demonstrated in rat glomeruli and human mesangial cells that the binding sites for losartan or EXP-985, both AT1RA, have a substantially greater (not smaller, as one may predict from its inability to bind to non-AT1 receptors) density than AngII binding sites, suggesting that losartan does recognize more than just AT1 receptors (236,237). Moreover, the binding of these two ligands to rat glomeruli does not exhibit the same sensitivity to GTP and dithiothreitol (238). Furthermore, losartan *in vitro* at high concentrations causes intrinsic effects on glomeruli and mesangial cells, including glomerular and mesangial cell contractility, stimulation of cytosolic calcium, and disorganization of the α -actin filament bundles in mesangial cells (239).

Another difference between ACEi and AT1RA is that AT1RA increases renin and AngII levels, leading to activation of non-AT1 receptors. However, the significance of non-AT1 receptors in human disease has not been established. It should be noted that there have been no clinical data to indicate that AT1RA has a renoprotective effect as ACEi does. Recent studies in animal models of progressive renal failure have revealed that, at certain dosages, these agents are comparable in their protection of the kidney from structural derangement (117,146). Nevertheless, readers of this article are reminded of the recent and somewhat disappointing experience that the effect of ACEi in humans was found not to be as dramatically renoprotective as one may have hoped from the impressive effect of ACEi demonstrated in many rodent models. A more detailed discussion of this issue is available elsewhere (240). In that study, two schools of thought are presented on the basis of the available animal and human data that suggest that AT1RA, in contrast to ACEi, has less marked glomerular hemodynamic effects (241–243). Thus, because of the lesser glomerular pressure-lowering effect of AT1RA, (1) AT1RA may be less renoprotective than ACEi through a dampening of the potentially injurious effect of high glomerular pressure; or (2) AT1RA may not elicit the unwanted initial fall of GFR that is often followed by ACEi administration, and may therefore maintain a relatively high GFR, postponing the requirement of dialysis longer than that achievable with ACEi.

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al., U.S. Patent No. 5,071,861), and also has a patent application pending under the title of "Method and compositions for maintaining glomerular filtration rate while inhibiting extracellular matrix accumulation" (Kon V, et al., filed on July 25, 1994).

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