Angiotensin-Converting Enzyme Inhibition and Angiotensin II Antagonism in Nondiabetic Chronic Nephropathies

By Piero Ruggenenti

Angiotensin II (A II), the main effector of the renin angiotensin system (RAS), plays a central role in the hemodynamic and nonhemodynamic mechanisms of chronic renal disease and is currently the main target of interventions aimed to prevent the onset and progression of chronic nephropathies to end-stage renal disease (ESRD). In addition, to ameliorate glomerular hyperfiltration and size selectivity, reduce protein traffic and prevent glomerular and tubulointerstitial toxicity of ultrafiltered proteins, RAS inhibitors also limit the direct nephrotoxic effects of A II. Thus, both angiotension-converting enzyme (ACE) inhibitors (ACEi) and A II antagonists (ATA) exert a specific nephroprotective effect in both experimental and human chronic renal disease. This effect is time-dependent and is observed across degrees of renal insufficiency. Forced ACEi or ATA uptitration above doses recommended to control arterial hypertension and combined treatment with both agents allow optimization of A II inhibition and maximization of renoprotection. Multifactorial interventions combining RAS inhibition to treatments targeted also to non-RAS mechanisms could even achieve regression of glomerulosclerosis and chronic tubulointerstitial injury. Studies are needed to assess whether renal damage can be reverted to such a point that renal function could be fully prevented from worsening, and possibly improvement. The economic impact of even a partial improvement would be enormous. Moreover, chronic renal insufficiency is an independent risk factor for cardiovascular disease, and effective nephroprotection could also decrease the excess cardiovascular morbidity and mortality associated with chronic nephropathies. In patients with renal insufficiency, ACEi are even more cardioprotective than in those without and are well tolerated. Thus, RAS inhibitor therapy should be offered to all renal patients without specific contraindications, including those closer to renal replacement therapy.

ANGIOTENSIN II (A II), the main effector of the renin angiotensin system (RAS), plays a central role in the hemodynamic and nonhemodynamic mechanisms of chronic renal disease and is currently the main target of interventions aimed to prevent the onset and progression of chronic nephropathies to end-stage renal disease (ESRD).[1] In vivo, A II enhances the vascular tone of both afferent and efferent glomerular arterioles, modulating intraglomerular capillary pressure and glomerular filtration rate. A II exerts its vasoconstrictor effect predominantly on the postglomerular arterioles, thereby increasing the glomerular hydraulic pressure and the filtration fraction (glomerular hyperfiltration). High glomerular capillary pressure increases the radius of the pores in the glomerular membrane, thus impairing the size-selective function of the membrane to plasma macromolecules.[2] In isolated perfused kidneys, infusion of A II results in a loss of glomerular size selectivity and proteinuria, an effect that has been attributed not only to the hemodynamic activity of A II,[3] but also to its direct effect on the glomerular barrier.[4] Podocytes have a complex cytoskeleton with contractile properties, and there are A II receptors on their surface[5]; these findings have suggested that A II could alter perm-selective properties of the glomerular barrier by mediating contraction of the foot processes, ultimately changing slit diaphragm architecture and allowing proteins to escape more easily into the urinary space.[6] Evidence that A II depolarizes podocytes by opening a chloride conductance related to cytoskeleton through an AT_1 receptor is in line with such a possibility.[7] Increased glomerular permeability results in an abnormal protein trafficking through the glomerular capillary that contributes to progressive glomerular and tubulointerstitial damage, and eventually results in renal function loss and scarring. [8]

Glomerular Toxicity of Ultrafiltered Proteins

Recent data are in support of the possibility that the excessive protein load of the cells can be a factor underlying progressive podocyte injury.[9] Signs of enhanced uptake of plasma proteins by podocytes, as assessed by immunofluorescence analysis of IgG and complement C3, were found in

From the Department of Medicine and Transplantation, Ospedali Riuniti, Bergamo-Mario Negri Institute for Pharmacological Research, Bergamo, Italy, and the Department of Renal Medicine, Clinical Research Center for Rare Diseases "Aldo e Cele Daccò," Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

Address reprint requests to Piero Ruggenenti, MD, Mario Negri Institute for Pharmacological Research, Via Gavazzeni, 11, 24125 Bergamo, Italy. E-mail: pruggenenti@ospedaliriuniti.bergamo.it

0270-9295/04/2402-0009$30.00/0

doi:10.1016/j.semnephrol.2003.11.002

remnant kidneys of rats with 5/6 renal mass reduction at 7 days after surgery in a very early stage of disease. The granular intracellular pattern was entirely consistent with accumulation of proteins by endocytosis. By dual staining of sections of kidneys taken at 14 days after surgery, the abnormal expression of desmin, a marker of podocyte injury, was confined to the podocytes showing intracellular staining for plasma proteins. In addition, protein-laden podocytes showed loss of expression of synaptopodin, an actin-associated molecule first detectable during foot process formation and thus an indicator of differentiated phenotype of the cell. These data were taken to suggest that the enhanced endocytosis of protein could concur to the perturbation of podocyte function that is currently recognized to play a major role in generating adhesive lesions and sclerosis. A causal link between protein load and podocyte dysfunction indeed was established by findings that the exposure of cultured podocytes to albumin (10 mg/mL) induced both loss of synaptopodin staining and expression and release of TGF-β1, a major stimulus for extracellular matrix production in the glomerulus. Moreover, the conditioned medium of IgG-laden podocytes induced the expression of the myofibroblast-associated molecule alpha-smooth muscle actin in cultured mesangial cells. Such response was inhibited by the addition of neutralizing anti-TGF-β1 antibody.[9]

TUBULOINTERSTITIAL TOXICITY OF ULTRAFILTERED PROTEINS

Pioneering studies in rats with age-related proteinuria[10] or with adriamycin-induced nephrosis[11] found that protein reabsorption droplets accumulate in the proximal tubular cells. Evidence that protein accumulation was associated with focal breaks of tubular basement membranes and extravasation of the tubular content in the renal interstitium suggested that plasma proteins could contribute to the tubulointerstitial damage so frequently observed in animals or humans with long-lasting, proteinuric nephropathies.

Both in vitro and in vivo, protein overload causes increased production of inflammatory mediators such as endothelin-1, monocyte chemoattractant protein-1 (MCP-1), RANTES (regulated upon activation normal T-cell expressed and secreted), a chemotactic cytokine for monocytes and memory T cells, and osteopontin.[12] The molecular mechanisms that lead to chemokine overexpression is mediated by activation of the NF-κB transcription factor. That promotes nuclear translocation of the DNA.[13,14] There is in vitro evidence that albumin and IgG caused a dose-dependent increase in NF-κB activation in proximal tubular cells, an event that is followed by upregulation of RANTES and MCP-1.[15,16] In specimens of renal biopsies of patients with severe proteinuria, NF-κB activation has been shown in tubular cells, concomitant to upregulation of proinflammatory chemokines.[17]

Cytokines, growth factors, and vasoactive substances can result in abnormal accumulation of extracellular matrix collagen, fibronectin, and other components that are responsible for interstitial fibrosis. The proinflammatory mediators promote local recruitment of macrophages and lymphocytes, which in turn can stimulate the transformation of interstitial cells into myofibroblasts.[18] Proximal tubular epithelial cells can interact with interstitial fibroblasts to promote fibrogenesis through release of profibrogenic molecules.[19]

Protein overload could also lead to an in situ activation of the complement system in proximal tubular cells associated with marked cytoskeleton alterations, increased production of superoxide anion and hydrogen peroxide, synthesis of proinflammatory cytokines, and transmigration of T cells across tubular epithelial cells.[20] Moreover, ultrafiltration of complement factors across the altered glomerular barrier could lead to complement C3 deposition and membrane attack complex formation (C5b-9) on the luminal side as well as C3 accumulation within proximal tubular cells.[12] These findings, combined with evidence that congenital absence of C6 limits interstitial inflammation and preserves renal function and structure in several proteinuric conditions,[21] suggest a major role of the complement system in the pathogenesis of proteinuria-induced interstitial damage.

DIRECT TOXICITY OF ANGIOTENSIN II

A II has an intrinsic toxicity that is independent of, and additional to, the nephrotoxic effects of increased protein traffic. AII modulates renal cell growth, which in turn could contribute to tubulointerstitial injury.[22] Increased expression of *c-fos* and *Egr-1*, the immediate early genes whose activation precedes cell proliferation, has been shown in proximal tubular cells exposed to AII.[23] The peptide, acting through AII type 1 receptors, also

induces hypertrophy in tubular cells by upregulating the gene for transforming growth factor β1 (TGF-β1), which in turn leads to increased synthesis of collagen type IV.[24] Remodeling of the interstitial architecture could also occur as a result of transformation of tubular cells, an additional event promoted by the enhanced synthesis of TGF-β1 stimulated by A II.[25]

A II also stimulates the production of plasminogen activator inhibitor-1 (PAI-1) and could therefore further increase the accumulation of the extracellular matrix through inhibition of its breakdown by matrix metalloproteinases, which require the conversion to an active form by plasmin.[26] By stimulating macrophage activation and phagocytosis, A II could enhance the inflammatory component associated with chronic renal injury.[27] A II upregulates genes and stimulates secretion of peptides with chemotactic and vasoactive properties.[28] In experimental animals, repeated infusions of A II cause interstitial fibrosis and lead to the deposit of type IV collagen, a process that suggests the morphogenic effect of A II on tubulointerstitial structure.[29] Studies in a protein overload model of nephropathy[30] allowed to dissect the relative contribution of A II and proteinuria on chronic renal damage in animals with targeted gene deletion of the A II type 1A receptor (AT1 $-/-$) as compared with wild-type mice (AT1 $+/+$). Normal animals not exposed to overload proteinuria acted as controls. AT1 $-/-$ animals developed proteinuria, renal failure, and glomerular sclerosis, although to a lesser degree than AT1 $+/+$ animals. In both models, renal endothelin-1 expression and synthesis was increased as compared with normal controls. These data confirm that both AII and plasma proteins have an intrinsic renal toxicity that is maximized when the two factors can play in combination.[30]

ANGIOTENSIN II INHIBITION AND NEPHROPROTECTION

Animal Studies

Evidence that A II blockade with an angiotensin-converting enzyme inhibitor (ACEi) reduced proteinuria and slowed renal damage in a number of animal models of chronic renal disease,[31,32] offered the opportunity, for the first time, to devise a treatment strategy that was not limited to passively accompany patients to their destiny of dialysis, but was aimed to preserve renal function as long as possible.

The antiproteinuric effect of A II inhibition has been initially attributed to the reduction of glomerular hypertension, but a direct effect on glomerular membrane perm selectivity to macromolecules has been also demonstrated.[32] In in vitro and in vivo experiments, ACEi prevent the expression of inflammatory mediators such as NF-κB, RANTES, MCP-1, and insulin-like growth factor.[33] This could result both from decreased exposure of glomerular and tubular cells to toxic effects of proteins and from direct inhibition of the proinflammatory properties of A II. In the remnant kidney model, the ACEi treatment limited the upregulation of TGF-β1 in podocytes, as well as the abnormal expression of alpha-smooth muscle actin in mesangial cells.[33] The development of a new class of RAS inhibitors, such as the angiotensin receptor blockers (ARBs), has opened the perspective of novel strategies to achieve renoprotection. Indeed, several studies in different models of chronic renal disease uniformly found that ARBs could shear with ACEi a similar antiproteinuric and renoprotective effect.[34]

Human Studies

Over the last decade, several clinical trials have evaluated whether the encouraging results achieved with RAS inhibitors in experimental animals translated in a real clinical benefit for humans with progressive nephropathies. In one of these trials, ACEi decreased the risk of doubling serum creatinine by 53%.[35] However, the large blood pressure difference between ACEi and placebo made it impossible to separate the beneficial effects of better blood pressure control from any other effect specific to the inhibitor. Evidence for a specific renoprotective effect of ACEi was provided by the Ramipril Efficacy in Nephropathy (REIN) study.[36-38] In this study, patients were randomly assigned to receive ramipril or conventional antihypertensive therapy to maintain diastolic blood pressure at 90 mm Hg or less. The trial was divided into two levels based on degree of baseline proteinuria (stratum 1: 1-3 g/24 hour and stratum 2 $>$ 3 g/24 hours). The stratum 2 arm was stopped early because of greater efficacy of ramipril on preserving glomerular filtration rate (GFR). Despite a virtual identical blood pressure control in the two treatment groups, the ramipril group showed a slower rate of

loss of GFR (mean monthly GFR decline 0.53 mL/min vs 0.88 mL/min) and a 50% lower incidence of ESRD as compared with controls.[36] Both effects were associated with a greater decrease in proteinuria (55% for ramipril vs no reduction for placebo). The rate of GFR decline was correlated negatively with the extent of proteinuria reduction[36] and positively with the level of residual proteinuria.[39] Of note, the renoprotective effect was seen across degrees of renal insufficiency, and patients in the lowest tertile of GFR (GFR 10-30 mL/min) also benefited from treatment with ACEI without a significant increase in the risk of hyperkalemia.[40] ACEi reduced the rate of decline of residual renal function even in patients with ESRD treated with peritoneal dialysis.[41]

The African-American Study of Kidney disease (AASK) found a similar renoprotective effect also in patients, eg, blacks with hypertensive renal disease, generally considered to poorly respond to ACEi therapy. Indeed, ramipril as compared with amlodipine decreased GFR decline by 36% and progression to clinical end points by 38%, a finding that led the Ethics Committee to prematurely stop the amlodipine arm of the trial.[42] At final analyses, ACEi retained a superior renoprotective effect also as compared with beta blockade with metoprolol.[43]

Prolonged ACEi therapy resulted in even more effective renoprotection. Nephrotic patients of the REIN study who, at completion of the core study, continued on ramipril for another 2 years (the REIN Follow-up Study), enjoyed a progressive decrease in GFR decline up to a rate approximately 1 mL/min/year, similar to that associated with normal aging.[37] After approximately 36 months, no more patients progressed to the point of requiring dialysis.[37] Even more surprisingly, GFR slopes in 16 of those patients progressively stabilized or were worsening so slowly that ESRD would be delayed beyond the patients' expected lifetime. Ten patients showed an improvement of GFR to the point that they might never reach ESRD.[44] On the contrary, patients originally on conventional treatment and switched to ramipril only on follow up, despite a substantially reduced GFR decline, continued to progress and, in some cases, developed ESRD. Thus, ESRD risk reduction went from 50% in the core (18 months) to 300% in the follow up (3-4 years) study, a finding consistent with a strongly time-dependent effect of ACEi.[44]

OPTIMIZED ANGIOTENSIN II INHIBITION TO HALT PROGRESSION

Although encouraging, evidence from both experimental studies and clinical trials suggests that RAS inhibition postpones ESRD in most cases, but definitively prevents dialysis only in a minority of patients. Indeed, as a result of the current lag time between starting treatment and achievement of remission, a substantial proportion of patients still progresses to ESRD before their renal function begins to stabilize. ACEi alone is sufficient to halt progression if therapy is started early, at GFRs still higher than 50 mL/min/1.73 m^2.[40] To achieve this target at more advanced stages, a multimodal approach based on maximized RAS inhibition is needed. First, a low-sodium diet could serve to activate the intrarenal RAS, which would maximize the response to ACEi or ATA. Diuretics could also achieve this, in particular when the response to RAS inhibition is blunted by sodium retention secondary to a high-sodium diet and/or severe renal insufficiency. However, maximized RAS inhibition mainly rests on the use of higher than antihypertensive doses of ACEi or ATA or of these two agents in combination.

High-Dose Angiotensin-Converting Enzyme Inhibitor Therapy

In Munich Wistar Fromter (MWF) rats with spontaneous disease, high-dose ACEi given late during the animal's life when animals were already heavily proteinuric decreased proteinuria and stopped the disease from progressing, as documented by a lower incidence of glomeruli affected by sclerotic lesions and less interstitial injury than untreated controls.[45] These data overall substantiated the results of previous morphologic studies showing that ACEi, at doses exceeding the antihypertensive doses, imparted an additional benefit to glomerular structure, reversing the early glomerular lesions but not the advanced ones.[46] Sclerosis was also remodeled in aging rats by inhibiting the renin–angiotensin system with an ATA given at high doses for 6 months.[47] The effect was attributed to the modulation of cortical cell turnover and inhibition of plasminogen activator-1 (PAI-1) expression.

In humans, lisinopril uptitrated to 40 mg/day (twice the standard antihypertensive dose for patients with normal renal function), despite no additional effects on blood pressure, further reduced

proteinuria and, importantly, dose-dependently ameliorated the dyslipidemia associated with the nephrotic syndrome.[48]

Combined Angiotension-Converting Enzyme Inhibitor and Angiotension Receptor Blockade Therapy

Complementary or alternative to forced ACEi or ATA up titration is combined treatment with both agents.[49,50] The combination of an ACEi and ATA has been suggested as a way to maximize RAS blockade by affecting both the bioavailability of A II through ACEi and also by affecting its activity at the receptor level. ACEi have the additional properties of blocking the breakdown of bradykinin, a vasodilator that also stimulates nitric oxide production. ATA do not affect the activity of the AT-R2, which appears to be important in vasodilation. Moreover, they antagonize the activity of A II produced by non-ACEi-sensitive enzymes such as chymase and other serine proteases.[49,50] The combination of these two drugs could be a way to block the effects of A II at the AT-R1 level while achieving both increased bradykinin levels and activation of the AT-R2. This approach has recently offered a powerful tool to induce regression of renal disease at functional and structural levels. In a recently published study, the treatment with ACEI and ATA given to MWF rats during the interval between 25 and 40 weeks of age had remarkable effects.[51] Combined therapy completely reversed protein excretion and ameliorated renal plasma flow and the glomerular ultrafiltration coefficient. The reduction of the extent of existing structural damage was a key finding. Specifically, the percentage of glomeruli with sclerotic lesions affecting less than 25% of the tuft decreased in respect to baseline in the absence of increases in the percentage of glomeruli with more severe lesions. The degree of tubulointerstitial injury, including protein cast formation, macrophage infiltration, and type III collagen accumulation, was also reduced by treatment. In this model, the glomerular permselective dysfunction attributable to large, nonselective pores of the membrane precedes and could play a role in structural injury independently of increased glomerular capillary hydraulic pressure. Given the effect of A II to disrupt the permselective function of the glomerular-filtering barrier, the primary action of a drug of ameliorating the functional barrier, presumably at the podocyte level, could contribute to preventing the detrimental effects of proteinuria and chemokine stimulation. Dual- as compared with single-drug RAS blockade provided superior benefit and partial regression of tubulointerstitial injury also in another model of severe, progressive renal disease, passive Heymann nephritis in uninephrectomized rats.[52]

On clinical grounds several studies found more proteinuria reduction with combined therapy than with ACEi or ATA alone.[49,50] This effect, however, was almost invariably associated with more blood pressure reduction with combined therapy, which did not allow concluding on whether the superior antiproteinuric effect of combined therapy depended on more RAS inhibition rather than on more blood pressure reduction. To dissect the relative contribution of these two mechanisms, we recently compared the antiproteinuric effect of combined therapy with halved doses of benazepril and valsartan with the effect of full doses of both agents used alone.[53] The finding that combined therapy reduced proteinuria more effectively than the two agents alone at virtually identical levels of blood pressure control provided consistent evidence of the intrinsic renoprotective effect of combined RAS inhibition. The benefit of combined therapy was more consistent, and clinically relevant, in patients with more severe, nephrotic-range proteinuria. The superior, long-term renoprotective effect of combined versus single-drug RAS inhibition was confirmed by the results of the COOPERATE study.[54] This study included 263 patients with nondiabetic, proteinuric nephropathies randomized to 3-year treatment with 3 mg/day of trandolapril, 100 mg/day of losartan, or with halved doses of both drugs in combination. Eleven percent of patients on combination treatment reached the combined primary end point of doubling of serum creatinine concentration or ESRD compared with 23% of patients on trandolapril alone (hazard ratio 0.38; 95% confidence interval [CI] 0.18-0.63; $p = 0.018$) and 23% of those on losartan alone (0.40; 0.17-0.69; $p = 0.016$). Thus, combined therapy reduced progression to the end point by 95% CI, approximately 60% as compared with single ACEi or ATA treatment. The most striking difference in groups was the much more consistent proteinuria reduction (vs prerandomization values) on dual RAS blockade (76%) than on single ACEi (44%) or ATA (42%) treatment. The finding that the three treatment groups did not differ with respect to risk

factors and showed the same reductions in blood pressure, combined with evidence that improved kidney survival was strongly associated with more proteinuria reduction, led further support to the hypothesis that proteinuria reduction could have an important pathogenetic role in the renoprotective effect of (dual) RAS blockade. Consistently with short-term data,[53] the greater the proteinuria at baseline, the more was proteinuria reduction on follow up.[54] Thus, in line with post-hoc analyses of the REIN study,[36-40] patients with more severe disease at study entry and predicted to have a faster progression on follow up were those who finally benefited the most of renoprotective treatment. Hence, combined therapy was well tolerated, even in patients with advanced renal insufficiency, which provided further evidence that the practice of avoidance of ACEi, ATA, or both to prevent further renal impairment and hyperkalemia in patients closer to ESRD is no longer justified.[40] Although good, however, these results show that a substantial proportion of patients with chronic nephropathies still continues to progress even on combined treatment.

IMPLEMENTING ANGIOTENSIN II INHIBITION WITH A MULTIFACTORIAL INTERVENTION: A WAY TO ACHIEVE REGRESSION?

The RAS is the major, but not unique, determinant of progressive renal damage. Thus, targeting renoprotective therapy solely to the RAS might not be enough to achieve full remission/regression of chronic renal disease. Actually, experimental and human evidence is accumulating that both glomerulosclerosis and chronic tubulointerstitial injury, once developed, can be stabilized and even reverted when RAS inhibition is combined with treatments targeted also to non-RAS mechanisms. Thus, in analogy with other major medical conditions such as cancer or HIV infection multifactorial treatments could be required to definitely cure chronic nephropathies.

Experimental Studies

In an animal model of nephrotic syndrome, the accelerated passive Heyman nephritis, a lipid-lowering drug added to ACEi and ATA, further lessened the structural damage and ameliorated the outcome.[55] Combining ACEI, ATA, and statin was therapeutic when given between 2 and 10 months. The triple drug therapy, despite similar blood pressure control as compared with less effective treatments, led to reduction of urinary protein to normal values and full prevention of renal failure.

Reduction of intrarenal leukocyte accumulation and expression of TGF-β could concur to mediate the beneficial effects.[52] Like TGFβ, other chemokines and growth factors of tubular and/or inflammatory cell origin such as interleukin-1 and tissue plasminogen activator (tPA) contribute to the production and regulation of glomerular and interstitial extracellular matrix. Some of these factors might play interrelated actions possibly relevant to regression of lesions. This appears to be the case, for instance, in mice lacking tPA that were protected against tPA-induced MMP9 gene expression and renal fibrosis.[56] Thus, besides RAS-blocking agents and statins, other drugs such as TGF-β inhibitors,[57] vasopeptidase inhibitors,[58] and agents to block immune and inflammatory reactions such as mycophenolate mofetil,[59] complement inhibitory molecules,[60] and, in perspective, anti-TNF antibodies,[61] are candidate components of the pharmacologic cocktail aimed to achieve regression of the renal lesions.

Human Studies

Evidence that regression of renal progressive disease and of the underlying lesion is achievable in humans can only be indirect, but it is fairly consistent and encouraging. Clinical findings of reduction of proteinuria to $<$0.3 g/24 hours and increasing glomerular filtration rate indicate regression of proteinuric chronic nephropathy, possibly reflecting improvement of renal structural changes[62] (Table 1). Combined therapy with ACEI, ATA, diuretics, and statins blunted proteinuria and stabilized GFR for almost 10 years in a young girl with nephrotic-range proteinuria who might otherwise have required dialysis within months.[63] In a series of ours, 26 patients whose proteinuria had been at least 3 g for more than 6 months, despite ACEI therapy, were given a standardized multidrug treatment, including diuretics, ACEi, ATA, statins, and nondihidropyridine calcium channel blockers. Nineteen (73%) of these patients achieved full remission of proteinuria and their renal function stabilized over 24 months. Whether, in parallel with clinical remission, renal damage can also be reduced is still matter of investigation. In support is the evidence by repeated biopsy for a trend of renal damage to regress

Table 1. Definitions of Progression, Remission, and Regression of Proteinuric Chronic Nephropathies

	Residual Proteinuria (g/24 hours)	GFR Decline (mL/min/1.73 m^2/year)	Renal Structural Changes
Progression	≥1.0	>1.0*	Worsening
Remission	1.0–0.3	0.0–1.0*	Stable
Regression	<0.3	<0.0	Improving

* Physiological glomerular filtration rate decline associated with aging: 1.0 mL/min/1.73 m^2/year.

leading to less mesangial expansion, more open capillaries, and less interstitial fibrosis.[64] At least 10 years were needed to reverse the lesions, which is entirely consistent with the concept that the timing of institution of therapy, besides the drug doses and combinations, is critical in the human setting exactly like in the experimental animal.[65]

THE IMPLICATIONS OF ACHIEVING REGRESSION

Regression of lesions could have significant impact on progressive renal disease and its sequelae. One might wonder why we should pursue the goal of improving renal structure and function if we can already successfully stabilize the disease perhaps for a lifetime. First and most obviously, improving renal function can have a major effect in reducing the number of patients with chronic renal disease that progresses to ESRD. Any improvement of renal structure and function should also translate into less risk of ESRD for those who have less compromised renal function. This would apply both to young patients and to the elderly who could incur more critical renal and cardiovascular risks. The economic impact of even a partial improvement would be enormous, as documented by findings that a 30% reduction in the rate of GFR decline would translate in more than 60 billion saved for providing renal replacement therapy to patients progressing to ESRD in the United States by the year 2010.[66] Finally, in certain settings such as membranous nephropathy, focal and segmental sclerotic lesions predict worse prognosis. What would happen if we could revert them? Understanding the mechanisms by which a given lesion can regress and its relationship to function will be crucial to understanding the relevant renal cell biology and therapeutic targets. Once again, investigation in experimental models will prove indispensable to the new task and will clarify whether renal damage can be reverted to such a point that renal function could be fully prevented from worsening, and possibly improve.

CARDIOVASCULAR RISK AND CARDIOPROTECTION IN NONDIABETIC CHRONIC RENAL DISEASE

Cardiovascular Risk in Chronic Renal Disease

Even in the absence of classic risk factors such as hypertension, diabetes, dyslipidemia, and smoking, patients with renal disease are at increased risk of cardiovascular events.[67,68] Cardiovascular morbidity and mortality linearly correlate with serum creatinine concentration, and in patients with terminal renal failure could exceed that in the general population by 10 to 100-fold.[68] The Heart Outcomes Prevention Evaluation (HOPE) study found that in patients with increased cardiovascular risk, even mild increases in serum creatinine (1.4-2.3 mg/dL) and microalbuminuria were equally strong-related risk predictors and were independent of each other. Patients with both risk factors had an incidence of cardiovascular events comparable to that in subjects with known coronary ischemic disease.[69]

Several factors have been claimed to explain this strong renal–cardiovascular association. Renal insufficiency and proteinuria can play a direct pathogenetic role in the onset and progression of atherosclerotic disease and can also amplify the effects of classic risk factors.[70] In particular, arterial hypertension (especially overnight),[71] dyslipidemia,[72] hyperhomocysteinemia,[73] and increased insulin resistance[74] could promote atherosclerosis and, in addition to cardiovascular remodeling,[72] could increase the cardiovascular risk even in the very early stages of chronic renal disease. Moreover, microalbuminuria is considered to reflect a generalized endothelial dysfunction that could independently contribute to macrovascular disease.[75] Finally, there is evidence that RAS activation could

be an independent risk factor for both renal and cardiovascular disease.[76]

Angiotensin II Inhibition and Cardioprotection in Chronic Renal Disease

Evidence that excess cardiovascular risk is associated with renal disease and that this excess could be, at least in part, associated with increased RAS activity,[76] provides a further, strong rationale to RAS inhibition therapy in patients with chronic nephropathies. Although ad hoc studies are missing, post-hoc analyses of the HOPE trial,[69] which included almost 1000 patients with mild–moderate renal insufficiency, allowed to evaluate the impact of renal function on the cardioprotective effects of ACEi therapy. Actually, ramipril uniformly decreased the overall cardiovascular risk across quartiles of basal serum creatinine and reduced overall mortality, cardiovascular mortality, and hospitalization for heart failure even more effectively in patients with serum creatinine $\geq$1.4 mg/dL than in the overall study population. On the same line, in the renal and cardiovascular treatment program introduced in late 1995 into an high-risk Australian Aboriginal community, ACEi therapy resulted in a 50% reduction in the rate of natural (mostly cardiovascular) deaths and 57% reduction in ESRD events.[77] A large part of renal and cardiovascular events occurred in subjects with evidence of renal disease (macroalbuminuria), and the beneficial effect of ACEi inhibitor therapy was largely driven by the remarkable risk reduction achieved in this subset of patients.[77] Despite these encouraging results, many physicians still have safety concerns about using RAS inhibitors in patients with renal insufficiency. However, both the REIN[36-38] and the HOPE[78] studies failed to detect any association between renal insufficiency and premature ramipril withdrawal because of adverse events. In particular, in the HOPE study, the incidence of ramipril-related symptomatic hypotension, cough, and angioedema was independent of basal serum creatinine levels. Moreover, at comparable levels of serum creatinine, the incidence of premature withdrawal because of hyperkalemia or worsening renal function was comparable on ramipril and on placebo. Thus, in patients with renal insufficiency, ACEi therapy is even more cardioprotective than in the general population and is well tolerated. Thus, it should not be withheld simply because of a moderate ($<$30%) elevation in serum creatinine concentration. Higher increases should arise the suspicion of a concomitant ischemic kidney disease or of an overzelous diuretic therapy resulting in decreased effective arterial volume and RAS activation. Response to treatment is less predictable in renal patients with congestive heart failure. In most cases, the improvement in systemic hemodynamics achieved by RAS inhibitor therapy could also result in an improvement in kidney function. However, when kidney perfusion and ultrafiltration are largely dependent on an activated (intrarenal) RAS system such as in patients with severe cardiac dysfunction and remarkably decreased effective arterial volume, RAS inhibition could result in kidney hypoperfusion and dysfunction. In most cases, however, this is a transient effect, and empiric downtitration of the ACEi or ATA dose and/or of concomitant diuretic therapy could help achieve a balance between the systemic and renal effects of ACE inhibition that could result in improved hemodynamics and kidney function.[79] Of note, the long-term benefit of RAS inhibition therapy appears similar in patients with and without substantial elevations in serum creatinine levels.[79]

REFERENCES

1. Aros C, Remuzzi G: The renin–angiotensin system in progression, remission and regression of chronic nephropathies. J Hypertens 20:S45-S53, 2002 (suppl)

2. Yoshioka T, Rennke HG, Salant DJ, et al: Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: A study in early passive Heymann nephritis. Circ Res 61:531-538, 1987

3. Bohrer MP, Deen WM, Robertson CR, Brenner BM: Mechanism of angiotensin II-induced proteinuria in the rat. Am J Physiol 233:F13-F21, 1977

4. Lapinski R, Perico N, Remuzzi A, et al: A II modulates glomerular capillary permselectivity in rat isolated perfused kidney. J Am Soc Nephrol 7:653-660, 1996

5. Kritz W, Hackenthal E, Nobiling R, et al: A role for podocytes to counteract capillary wall distension. Kidney Int 45:369-376, 1994

6. Shake JG, Brandt RC, Daniels BS: A II induces actin polymerization within the glomerular filtration barrier: Possible role in the local regulation of ultrafiltration. J Am Soc Nephrol 3:568, 1992 (abstract)

7. Gloy J, Henger A, Fischer K-G, et al: A II depolarizes podocytes in the intact glomerulus of the rat. J Clin Invest 99:2772-2781, 1997

8. Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. N Engl J Med 339:1448-1456, 1998

9. Abbate M, Zoja C, Morigi M, et al: Transforming growth factor-beta1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: A central pathway in progressive glomerulosclerosis. Am J Pathol 161:2179-2199, 2002

10. Bertani T, Zoja C, Abbate M, et al: Age-related nephrop-

athy and proteinuria in rats with intact kidneys exposed to diets with different protein content. Lab Invest 60:196-204, 1989

11. Bertani T, Cutillo F, Zoja C, et al: Tubulointerstitial lesions mediate renal damage in adriamycin glomerulopathy. Kidney Int 30:488-496, 1986

12. Zoja C, Morigi M, Remuzzi G: Proteinuria and phenotypic change of proximal tubular cells. J Am Soc Nephrol 14:S36-S41, 2003

13. Barnes PJ, Karin M: Nuclear factor-kB—A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336:1066-1071, 1997

14. Baldwin AS: The transcription factor NF-kB and human disease. J Clin Invest 107:3-11, 2001

15. Zoja C, Donadelli R, Colleoni S, et al: Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kB activation. Kidney Int 53:1608-1615, 1998

16. Morigi M, Macconi D, Zoja C, et al: Protein overload-induced NF-kB activation in proximal tubular cells requires H202 through a PKC-dependent pathway. J Am Soc Nephrol 13:1179-1189, 2002

17. Mezzano SA, Barria M, Droguett MA, et al: Tubular NF-kB and AP-1 activation in human proteinuric renal disease. Kidney Int 60:1366-1377, 2001

18. Eddy AA: Role of cellular infiltrates in response to proteinuria. Am J Kidney Dis 37:S25-S29, 2001 (Suppl 2)

19. Johnson DW, Saunders HJ, Baxter RC, et al: Paracrine stimulation of human renal fibroblasts by proximal tubule cells. Kidney Int 54:747-757, 1998

20. Zoja C, Benigni A, Remuzzi G: Cellular responses to protein overload: Key event in renal disease. Curr Opin Nephrol Hypertens 13:31-37, 2004

21. Hsu SI-H, Couser WG: Chronic progression of tubulointerstitial damage in proteinuric renal disease is mediated by complement activation: A therapeutic role for complement inhibitors? J Am Soc Nephrol 14:S186-S191, 2003

22. Wolf G: A II as a mediator of tubulointerstitial injury. Nephrol Dial Transplant 15:61-63, 2000 (suppl)

23. Wolf G, Ziyadeh F: Renal tubular hypertrophy induced by A II. Semin Nephrol 17:448-454, 1997

24. Wolf G, Kalluri R, Ziyadeh F, et al: A II induces alpha3 (IV) collagen expression in cultured urine proximal tubular cells. Proc Assoc Am Physic 11:357-364, 1999

25. Strutz F, Muller GA: Interstitial pathomechanisms underlying progressive tubulointerstitial damage. Kidney Blood Press Res 22:71-80, 1999

26. Baricos WH, Cortez SL, el-Dahr SS, et al: ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. Kidney Int 47:1039-1047, 1995

27. Keane WF, Raij L: Relationship among altered glomerular barrier permselectivity. A II, and mesangial uptake of macromolecules. Lab Invest 52:599-604, 1985

28. Egido J: Vasoactive hormones and renal sclerosis. Kidney Int 49:578-597, 1996

29. Okada H, Danoff T, Kalluri R, et al: Early role of Fsp 1 in epithelial–mesenchymal transformation. Am J Physiol 273: F563-F574, 1997

30. Benigni A, Corna D, Zoja C, et al: Targeted deletion of Angiotensin II type 1 receptor does not protect mice from progressive nephropathy of overload proteinuria. J Am Soc Nephrol 13:341(abstract), 2002

31. Zatz R, Dunn BR, Meyer TW, et al: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest 77:1925-1930, 1986

32. Remuzzi A, Puntorieri S, Battaglia C, et al: Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. J Clin Invest 85:541-549, 1990

33. Donadelli R, Abbate M, Zanchi C, et al: Protein traffic activates NF-kB gene signaling and promotes MCP-1-dependent interstitial inflammation. Am J Kidney Dis 36:1226-1241, 2000

34. Taal MW, Brenner BMRenoprotective benefits of RAS inhibitionFr**o** ACEi to angiotensin II antagonists: Kidney Int 57:1803-1817, 2000

35. Maschio G, Alberti D, Janin G, et al: Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal I Study Group. N Engl J Med 334:939-945, 1996

36. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia): Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 349:1857-1863, 1997

37. Ruggenenti P, Perna A, Gherardi G, et al, on behalf of Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN): Renal function and requirement for dialysis in chronic nephropathy patients on long-term ramipril: REIN follow-up trial. Lancet 352:1252-1256, 1998

38. Ruggenenti P, Perna A, Gherardi G et al: Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 354:359-364, 1999

39. Ruggenenti P, Perna A, Remuzzi G, on behalf of the Investigators of the GISEN Group: Retarding progression of chronic renal disease: The neglected issue of residual proteinuria. Kidney Int 63:2254-2261, 2003

40. Ruggenenti P, Perna A, Remuzzi G: ACE inhibitors to prevent end-stage renal disease: When to start and why possibly never to stop: A post hoc analysis of the REIN trial result. Ramipril Eficacy in Nephropathy. J Am Soc Nephrol 12:2832-2837, 2001

41. Kam-Tao P, Chow K-M, Wong T Y-H, Leung C-B, Szeto C-C: Effects of an angiotensin converting enzyme inhibitor on residual renal function in patients receiving peritoneal dialysis. Ann Intern Med 139:105-112, 2003

42. Agodoa LY, Appel L, Bakris GL, et al: African American Study of Kidney Disease and Hypertension (AASK) Study Group: Effect of ramipril vs amlodipine on renal outcomes in hypertensive nephrosclerosis: A randomized controlled trial. JAMA 285:2719-2728, 2001

43. Wright JT, Bakris G, Green T, et al: African American Study of Kidney Disease and Hypertension (AASK) Study Group: Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: Results from the AASK trial. JAMA 288:2421-2431, 2002

44. Ruggenenti P, Perna A, Benini R, et al: In chronic nephropathies prolonged ACE inhibition can induce remission: Dynamics of time-dependent changes in GFR. J Am Soc Nephrol 10:997-1006, 1999

45. Remuzzi A, Fassi A, Bertani T, et al: ACE inhibition induces regression of proteinuria and halts progression of renal

damage in a genetic model of progressive nephropathy. Am J Kidney Dis 34:626-632, 1999

46. Ikoma M, Kawamura T, Kakinuma Y, et al: Cause of variable therapeutic efficiency of angiotensin converting enzyme inhibitor on glomerular lesions. Kidney Int 40:195-202, 1991

47. Ma L-J, Nakamura S, Whitsitt JS, et al: Regression of sclerosis in aging by an angiotensin inhibition-induced decrease in PAI-1. Kidney Int 58:2425-2436, 2000

48. Ruggenenti P, Mise N, Pisoni R, et al: Diverse effects of increasing Lisinopril doses on lipid abnormalities in chronic nephropathies. Circulation 107:586-592, 2003

49. Taal MW, Brenner BM: Combination ACEi and ARB therapy: Additional benefit in renoprotection? Curr Opin Nephrol Hypertens 11:377-381, 2002

50. Ruggenenti P, Remuzzi G. Is therapy with combined ACE inhibitor and angiotensin receptor antagonist the new "gold standard" of treatment for non-diabetic chronic proteinuric nephropathies? Nephrology (in press)

51. Remuzzi A, Gagliardini E, Donadoni A, et al: Effect of A II antagonism on the regression of kidney disease in the rat. Kidney Int 62:885-894, 2002

52. Zoja C, Corna D, Camozzi D, et al: How to fully protect the kidney in a severe model of progressive nephropathy: A multidrug approach. J Am Soc Nephrol 13:2898-2908, 2002

53. Campbell R, Sangalli F, Perticucci E, et al: Effects of combined ACE inhibitor and angiotensin II antagonist treatment in human chronic nephropathies. Kidney Int 63:1094-1103, 2003

54. Nakao N, Yoshimura A, Morita H, et al: Combination treatment of angiotensin II receptor blocker and angiotensin converting enzyme inhibitor in non diabetic renal disease (COOPERATE): A randomized controlled trial. Lancet 361: 117-124, 2003

55. Zoja C, Corna D, Rottoli D, et al: Effect of combining ACE inhibitor and statin in severe experimental nephropathy. Kidney Int 61:1635-1645, 2002

56. Yang J, Shultz RW, Mars WM, et al: Disruption of tissue-type plasminogen activator gene in mice reduces renal interstitial fibrosis in obstructive nephropathy. J Clin Invest 110:1525-1538, 2002

57. Ziyadeh FN, Hoffman BB, Cheol Han D, et al: Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-β antibody in *db/db* diabetic mice. Proc Natl Acad Sci USA 97:8015-8020, 2000

58. Taal MW, Nenov VD, Wong W, et al: Vasopeptidase inhibition affords greater renoprotection than angiotensin-converting enzyme inhibition alone. J Am Soc Nephrol 12:2051-2059, 2001

59. Remuzzi G, Zoja C, Gagliardini E, et al: Combining an antiproteinuric approach with micophenolate mofetil fully suppresses progressive nephropathy of experimental animals. J Am Soc Nephrol 10:1542-1549, 1999

60. Nangaku M, Pippin J, Couser WG: C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. J Am Soc Nephrol 13:928-936, 2002

61. Muller DN, Shagdarsuren E, Park J-K, et al: Immunosuppressive treatment protects against A II-induced renal damage. Am J Pathol 161:1679-1693, 2002

62. Ruggenenti P, Schieppati A, Remuzzi G: Progression, remission, regression of chronic renal diseases. Lancet 357: 1601-1608, 2001

63. Ruggenenti P, Brenner BM, Remuzzi G: Remission achieved in chronic nephropathy by a multidrug approach targeted at urinary protein excretion. Nephron 88:254-259, 2001

64. Fioretto P, Steffes MW, Sutherland DE, et al: Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med 339:69-75, 1998

65. Perico N, Amuchastegui CS, Colosio V, et al: Evidence that an angiotensin converting enzyme inhibitor has a different effect on glomerular injury according to the different phase of the disease at which the treatment is started. J Am Soc Nephrol 5:1139-1146, 1994

66. Trivedi HS, Pang MMH, Campbell A, et al: Slowing the progression of chronic renal failure: Economic benefits and patients' perspectives. Am J Kidney Dis 39:721-729, 2002

67. Hall WD: Abnormalities of kidney function as a cause and a consequence of cardiovascular disease. Am J Med Sci 317:176-182, 1999

68. Parfrey PS, Foley RN: The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol 10:1606-1615, 1999

69. Mann JFE, Gestein HC, Pogue J, et al: Renal insufficiency as a predictor of cardiovascular outcomes and the impact of Ramipril: The HOPE randomized trial. Ann Intern Med 134:629-636, 2001

70. Baigent C, Burbury K, Wheeler D: Premature cardiovascular disease in chronic renal failure. Lancet 356:147-152, 2000

71. Sdevanski A, Schmidt KG, Waldherr R, et al: Early increase in blood pressure and diastolic left ventricular malfunction in patients with glomerulonephritis. Kidney Int 50: 1321-1326, 1996

72. Kronenberg F, Kuen E, Ritz E, et al: Apolipoprotein A-IV serum concentrations are elevated in patients with mild and moderate renal failure. J Am Soc Nephrol 11:105-115, 2000

73. Jungers P, Joly D, Massy Z, et al: Sustained reduction of hyperhomocysteinaemia with folic acid supplementation in predialysis patients. Nephrol Dial Transplant 14:2903-2906, 1999

74. Fliser D, Pacini G, Engelleiter R, et al: Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int 53:1343-1347, 1998

75. Gall M-A, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving H-H: Albuminuria and poor glycemic control predict mortality in NIDD? Diabetes 44:1303-1309, 1995

76. Yusuf S, Ionn E: Anti-ischemic effects of ACE-inhibitors: Review of current clinical evidence and ongoing trials. Eur Heart J 19:J36-J44, 1998

77. Hoy WE, Wang Z, Baker PRA, Kelly AM: Secondary prevention of renal and cardiovascular disease: Results of a renal and cardiovascular treatment program in an Australian Aboriginal community. J Am Soc Nephrol 14:S178-S185, 2003

78. The Heart Outcomes Prevention Evaluation Study Investigators: Effects of angiotensin converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. N Engl J Med 342:145-153, 2000

79. Shlipak MG: Pharmacotherapy for heart failure in patients with renal insufficiency. Ann Intern Med 138:917-924, 2003