The way to cardio-renal protection in non-diabetic chronic nephropathies

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Abstract

Angiotensin II (AII), the main effector of the Renin Angiotensin System (RAS), plays a central role in the hemodynamic and non-hemodynamic 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 traffick and prevent glomerular and tubulo-interstitial toxicity of ultrafiltered proteins, RAS inhibitors also limit the direct nephrotoxic effects of AII. Thus, both ACE inhibitors (ACEi) and AII 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 up-titration above doses recommended to control arterial hypertension and combined treatment with both agents allow to optimise A II inhibition and maximize renoprotection. Multifactorial interventions combining RAS inhibition to treatments targeted also to non-RAS mechanisms may even achieve regression of glomerulosclerosis and chronic tubulo-interstitial injury. Studies are needed to assess whether renal damage can be reverted to such a point that renal function may be fully prevented from worsen, and possibly improve. 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 may 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.

Key words: cardio-renal protection, non-diabetic chronic nephropathies.

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Received 6.05.2004 Accepted 11.06.2004

The key role of angiotensin II in the pathogenesis and progression of chronic renal disease

Angiotensin II (A II), the main effector of the Renin Angiotensin System (RAS), plays a central role in the hemodynamic and non-hemodynamic 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, AII enhances the vascular tone of both afferent and efferent glomerular arterioles, modulating intraglomerular capillary pressure and glomerular filtration rate. AII 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 AII [3], but also to its direct effect on the glomerular barrier [4]. Podocytes have a complex cytoskeleton with contractile properties, and there are AII receptors on their surface [5]: these findings have suggested that AII may 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 via an AT1 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 tubulo-interstitial 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 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 may 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-loaden 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].

Tubulo-interstitial 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 led to suggest that plasma proteins may contribute to the tubulo-interstitial 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 over-expression is mediated by NF-kB, a 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-kB activation in proximal tubular cells, an event that is followed by up-regulation of RANTES and MCP-1 [15,16]. In specimen of renal biopsies of patients with severe proteinuria, NF-kB activation has been shown in tubular cells, concomitant to up-regulation of pro-inflammatory chemokines [17].

Cytokines, growth factors and vasoactive substances may result in abnormal accumulation of extracellular matrix collagen, fibronectin and other components which are responsible for interstitial fibrosis. The pro-inflammatory 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 via release of profibrogenic molecules [19].

Protein overload may 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 may 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 to 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

AII 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 may 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 up-regulating 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 may also occur as a result of transformation of tubular cells, an additional event promoted by the enhanced synthesis of TGF-ß1 stimulated by AII [25].

A II also stimulates the production of plasminogen activator inhibitor-1 (PAI-1), and may therefore further increase the accumulation of the extracelular 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, AII may enhance the inflammatory component associated with chronic renal injury [27]. AII up-regulates genes and stimulate secretion of peptides with chemotactic and vasoactive properties [28]. In experimental animals, repeated infusions of AII cause interstitial fibrosis and lead to the deposit of type IV collagen, a process that suggests the morphogenic effect of AII on tubulointerstitial structure [29]. Studies in a protein overload model of nephropathy [30] allowed to dissect the relative contribution of AII and proteinuria on chronic renal damage in animals with targeted gene deletion of the AII type 1A receptor (AT1-/-) as compared to wildetype 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 ET1 expression and synthesis was increased as compared to normal controls. These data confirm that both A II and plasma proteins have an intrinsic renal toxicity that is maximized when the two factors may 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 which was not limited to passively accompain patients to their destiny of dialysis, but was aimed to preserve renal function as long as possible.

The antiproteinuric effect of AII inhibition has been initially attributed to the reduction of glomerular hypertension, but a direct effect on glomerular membrane permselectivity to macromolecules has been also demonstrated [32]. In in vitro and in vivo experiments ACEi prevent the expression of inflammatory mediators such as NF-kB, RANTES, MCP-1, and insulin-like growth factor [33]. This may 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 ACE inhibitor 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 may 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 gm/24 hour and stratum 2>3 gm/24 hours). The stratum 2 arm was stopped early because of greater efficacy of ramipril on preserving 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 to 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 to 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 – e.g. African-Americans with hypertensive renal disease – generally considered to poorly respond to ACEi therapy. Indeed, ramipril as compared to 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 to 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 two 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 about 36 months no more patient progressed to the point of requiring dialysis [37]. Even more surprisingly, GFR slopes in sixteen of those patients progressively stabilized or were worsening so slowly that ESRD would be delayed beyond the patients' expected life time. 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, evidences from both experimental studies and clinical trials suggest that RAS inhibition postpones ESRD in most cases, but definitively prevent dialysis only in a minority of patients. Indeed, due to 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 hearly, 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 may serve to activate the intrarenal RAS, which would maximize the response to ACEi or ATA. Diuretics may also achieve this, in particular when the response to RAS inhibition is blunted by sodium retention secondary to 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 ACE 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 morphological 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 six 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 up-titrated 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 ACEi and ARB 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 may 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 may play 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 drug of ameliorating the functional barrier, presumably at the podocyte level, could contribute to prevent the detrimental effects of proteinuria and chemokine stimulation. Dual as compared to 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 the clinical ground, 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]. 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 vs single drug RAS inhibition was confirmed by the results of the COOPERATE study [54]. This study included 263 patients with non-diabetic, 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 endpoint of doubling of serum creatinine concentration

Table 1. **Definitions of progression, remission and regression of proteinuric chronic nephropathies**

	Residual proteinuria (g/24 hours)	GFR decline (ml/min/1.73m2/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 GFR decline associated with aging: 1.0 ml/min/1.73m^2/year

or ESRD compared with 23 percent of patients on trandolapril alone (hazard ratio 0.38, 95% CI 0.18-0.63, p = 0.018) and 23 percent of those on losartan alone (0.40, 0.17-0.69, p = 0.016). Thus, combined therapy reduced progression to the endpoint by about 60% as compared to single ACEi or ATA treatment. The most striking difference in groups was the much more consistent proteinuria reduction (versus pre-randomization values) on dual RAS blockade (76%) than on single ACEi (44%) or ATA (42%) treatment. Finding that the three treatment groups did not differ with respect to risk factors and showed the same reductions in blood pressure, combined to evidence that improved kidney survival was strongly associated with more proteinuria reduction, led further support to the hypothesis that proteinuria reduction may 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 may 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 to treatments targeted also to non-RAS mechanisms. Thus – in analogy with other major medical conditions such as cancer or HIV infection – multi-factorial treatments may 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 to 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 may concur to mediate the beneficial effects [52]. Like TGF-β, 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 pharmacological 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/24h and increasing glomerular filtration rate indicate regression of proteinuric chronic nephropathy possibly reflecting improvement of renal structural changes [62] (*Tab. 1*). Combined therapy with ACEi, ATA, diuretics and statins blunted proteinuria and stabilized GFR for almost ten years in a young girl with nephrotic-range proteinuria who might otherwise have required dialysis within months [63]. In a series of ours, twenty-six 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 non-dihidropyridine 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 leading to less mesangial expansion, more open capillaries and less interstitial fibrosis [64]. At least ten years were needed to reverse the lesions, which is entirely consistent with the concept that the timing of institution of therapy, beside 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 may 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 may 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 that may incur in 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 $ billions saved for providing renal replacement therapy to patients progressing to ESRD in the US 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 may 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 may be fully prevented from worsen, and possibly improve.

Cardiovascular risk and cardioprotection in non-diabetic 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 may exceed that in the general population by ten to one hundred folds [68]. The HOPE study found that in patients with increased cardiovascular risk, even mild increases in serum creatinine (1.4 to 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 of subjects with known coronary ischemic disease [69].

Several factors have been claimed to explain this strong renal-cardiovascular association. Renal insufficiency and proteinuria may play a direct pathogenetic role in the onset and progression of atherosclerotic disease, and may also amplify the effects of classic risk factors [70]. In particular, arterial hypertension (especially overnight), [71], dyslipidemia [72], hyper-homocysteinemia [73], and increased insulin resistance [74], may promote atherosclerosis and, in addition to cardiovascular remodeling [72], may 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 may independently contribute to macrovascular disease [75]. Finally, there is evidence that RAS activation may 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 may 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 Heart Outcomes Prevention Evaluation (HOPE) trial [69], that included almost one thousand 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 rate of natural (mostly cardiovascular) deaths and 57% reduction in ESRD events [77]. 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 to use RAS inhibitors in patients with renal insufficiency. However, both the REIN [36-38] and the HOPE [78] study 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 may 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 may result in kidney hypoperfusion and dysfunction. In most cases, however, this is a transient effect and empirical down-titration of the ACEi or ATA dose and/or of concomitant diuretic therapy may help achieving a balance between the systemic and renal effects of ACE inhibition that may 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 Hyperten, 2002; 20: 45-53.
2. Yoshioka T, Rennke HG, Salant DJ. Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: A study in early passive Heymann nephritis. Circ Res, 1987; 61: 531-8.
3. Bohrer MP, Deen WM, Robertson CR, Brenner BM. Mechanism of angiotensin II-induced proteinuria in the rat. Am J Physiol, 1977; 233: 13-21.
4. Lapinski R, Perico N, Remuzzi A. A II modulates glomerular capillary permselectivity in rat isolated perfused kidney. J Am Soc Nephrol, 1996; 7: 653-60.
5. Kritz W, Hackenthal E, Nobiling R, Sakai T, Elger M, Hahnel B. A role for podocytes to counteract capillary wall distension. Kidney Int, 1994; 45: 369-76.
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, 1992; 3: 568.
7. Gloy J, Henger A, Fischer K-G. A II depolarizes podocytes in the intact glomerulus of the rat. J Clin Invest, 1997; 99: 2772-81.
8. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. N Engl J Med, 1998; 339: 1448-56.
9. Abbate M, Zoja C, Morigi M. 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, 2002; 161: 2179-99.
10. Bertani T, Zoja C, Abbate M, Rossini M, Remuzzi G. Age-related nephropathy and proteinuria in rats with intact kidneys exposed to diets with different protein content. Lab Invest, 1989; 60: 196-204.
11. Bertani T, Cutillo F, Zoja C. Tubulointerstitial lesions mediate renal damage in adriamycin glomerulopathy. Kidney Int, 1986; 30: 488-96.
12. Zoja C, Morigi M, Remuzzi G. Proteinuria and phenotypic change of proximal tubular cells. J Am Soc Nephrol, 2003; 14: 36-41.
13. Barnes PJ, Karin M. Nuclear factor-kB – A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med, 1997; 336: 1066-71.
14. Baldwin AS. The transcription factor NF-kB and human disease. J Clin Invest, 2001; 107: 3-11.
15. Zoja C, Donadelli R, Colleoni S. Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kB activation. Kidney Int, 1998; 53: 1608-15.
16. Morigi M, Macconi D, Zoja C. Protein overload-induced NF-kB activation in proximal tubular cells requires H2O2 through a PKC-Dependent pathway. J Am Soc Nephrol, 2002; 13: 1179-89.
17. Mezzano SA, Barria M, Droguett MA. Tubular NF-kB and AP-1 activation in human proteinuric renal disease. Kidney Int, 2001; 60: 1366-77.
18. Eddy AA. Role of cellular infiltrates in response to proteinuria. Am J Kidney Dis, 2001; 37(2): 25-29.
19. Johnson DW, Saunders HJ, Baxter RC, Field MJ, Pollock CA. Paracrine stimulation of human renal fibroblasts by proximal tubule cells. Kidney Int, 1998; 54: 747-57.
20. Zoja C, Benigni A, Remuzzi G. Cellular responses to protein overload: Key event in renal disease. Curr Opin Nephrol Hypertens (in press).
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, 2003; 14: 186-91.
22. Wolf G. A II as a mediator of tubulointerstitial injury. Nephrol Dial Transplant, 2000; 15: 61-3.
23. Wolf G, Ziyadeh F. Renal tubular hypertrophy induced by A II. Seminar Nephrol, 1997; 17: 448-54.
24. Wolf G, Kalluri R, Ziyadeh F. A II induces alpha3 (IV) collagen expression in cultured urine proximal tubular cells. Proc Ass Am Physic, 1999; 11: 357-64.
25. Strutz F, Muller GA. Interstitial pathomechanisms undelying progressive tubulointerstitial damage. Kidney Blood Press Re, 1999; 22: 71-80.
26. Baricos WH, Cortez SL, el-Dahr SS, Schnaper HW. ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. Kidney Int, 1995; 47: 1039-47.
27. Keane WF, Raij L. Relationship among altered glomerular barrier permselectivity, A II, and mesangial uptake of macromolecules. Lab Invest, 1985; 52: 599-604.
28. Egido J. Vasoactive hormones and renal sclerosis. Kidney Int, 1996; 49: 578-97.
29. Okada H, Danoff T, Kalluri R. Early role of Fsp 1 in epithelial-mesenchymal transformation. Am J Physiol, 1997; 273: 563-74.
30. Benigni A, Corna D, Zoja C. Targeted deletion of Angiotensin II Type 1 receptor does not protect mice from progressive nephropathy of overload proteinuria. J Am Soc Nephrol, 2002; 13: 341.
31. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest, 1986; 77: 1925-30.
32. Remuzzi A, Puntorieri S, Battaglia C. Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. J Clin Invest, 1990; 85: 541-9.
33. Donadelli R, Abbate M, Zanchi C, Corna D, Tomasoni S, Benigni A, Remuzzi G, Zoja C. Protein traffic activates NF-kB gene signaling and promotes MCP-1-dependent interstitial inflammation. Am J Kidney Dis, 2000; 36: 1226-41.
34. Taal MW, Brenner BM. Renoprotective benefits of RAS inhibition: fro ACEi to angiotensin II antagonists. Kidney Int, 2000; 57: 1803-17.
35. Maschio G, Alberti D, Janin G. 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, 1996; 334: 939-45.
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, 1997; 349: 1857-63.
37. Ruggenenti P, Perna A, Gherardi G, Gaspari F, Benini R, Remuzzi G 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, 1998; 352: 1252-6.
38. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M, Scolari F, Schena FP, Remuzzi G. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet, 1999; 354: 359-64.
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, 2003; 63: 2254-61.
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, 2001; 12: 2832-7.
41. Kam-Tao P, Chow K-M, Wong TY-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, 2003; 139: 105-12.
42. Agodoa LY, Appel L, Bakris GL. 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, 2001; 285: 2719-28.
43. Wright JT, Bakris G, Green T. 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, 2002; 288: 2421-31.

44. Ruggenenti P, Perna A, Benini R, Bertani T, Zoccali C, Maggiore Q, Salvadori M, Remuzzi G. In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. J Am Soc Nephrol, 1999; 10: 997-1006.

45. Remuzzi A, Fassi A, Bertani T. ACE inhibition induces regression of proteinuria and halts progression of renal damage in a genetic model of progressive nephropathy. Am J Kidney Dis, 1999; 34: 626-32.

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

47. Ma L-J, Nakamura S, Whitsitt JS, Marcantoni C, Davidson JM, Fogo AB. Regression of sclerosis in aging by an angiotensin inhibition-induced decrease in PAI-1. Kidney Int, 2000; 58: 2425-36.

48. Ruggenenti P, Mise N, Pisoni R, Arnoldi F, Pezzotta A, Perna A, Cattaneo D, Remuzzi G. Diverse effects of increasing Lisinopril doses on lipid abnormalities in chronic nephropathies. Circulation, 2003; 107: 586-92.

49. Taal MW, Brenner BM. Combination ACEi and ARB therapy: additional benefit in renoprotection? Curr Opin Nephrol Hypertens, 2002; 11: 377-81.

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. Effect of A II antagonism on the regression of kidney disease in the rat. Kidney Int, 2002; 62: 885-94.

52. Zoja C, Corna D, Camozzi D, Cattaneo D, Rottoli D, Batani C, Zonchi C, Abbate M, Remuzzi G. How to fully protect the kidney in a severe model of progressive nephropathy: a multidrug approach. J Am Soc Nephrol, 2002; 13: 2898-908.

53. Campbell R, Sangalli F, Perticucci E, Aros C, Viscarra C, Perna A, Remuzzi A, Bertocchi F, Fagiani L, Remuzzi G, Reggenenti P. Effects of combined ACE inhibitor and angiotensin II antagonist treatment in human chronic nephropathies. Kidney Int, 2003; 63: 1094-103.

54. Nakao N, Yoshimura A, Morita H. Combination treatment of angiotensin II receptor blocker and angiotensin converting enzyme inhibitor in non diabetic renal disease (COOPERATE): a randomized controlled trial. Lancet, 2003; 361: 117-24.

55. Zoja C, Corna D, Rottoli D, Cattaneo D, Zanchi C, Tomasoni S, Abbate M, Remuzzi G. Effect of combining ACE inhibitor and statin in severe experimental nephropathy. Kidney Int, 2002; 61: 1635-45.

56. Yang J, Shultz RW, Mars WM, Wegner RE, Li Y, Dai Ch, Nejak K, Liu Y. Disruption of tissue-type plasminogen activator gene in mice reduces renal interstitial fibrosis in obstructive nephropathy. JCI, 2002; 110: 1525-38.

57. Ziyadeh FN, Hoffman BB, Cheol Han D. 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. PNAS, 2000; 97: 8015-20.

58. Taal MW, Nenov VD, Wong W, Satyal SR, Sakharowa O, Choi JH, Troy JL, Brenner BM. Vasopeptidase inhibition affords greater renoprotection than angiotensin-converting enzyme inhibition alone. J Am Soc Nephrol, 2001; 12: 2051-9.

59. Remuzzi G, Zoja C, Gagliardini E. Combining an antiproteinuric approach with mycophenolate mofetil fully suppresses progressive nephropathy of experimental animals. J Am Soc Nephrol, 1999; 10: 1542-9.

60. Nangaku M, Pippin J, Couser WG. C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. J Am Soc Nephrol, 2002; 13: 928-36.

61. Muller DN, Shagdarsuren E, Park J-K, Dechend R, Mervaala E, Hampich F, Fiebeler A, Ju X, Finckenberg P, Theuer J, Viedt C, Kreuzer J, Heidecke H, Haller H, Zenke M, Luft FC. Immunosuppressive treatment protects against A II-induced renal damage. Am J Pathol, 2002; 161: 1679-93.

62. Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. Lancet, 2001; 357: 1601-8.

63. Ruggenenti P, Brenner BM, Remuzzi G. Remission achieved in chronic nephropathy by a multidrug approach targeted at urinary protein excretion. Nephron, 2001; 88: 254-9.

64. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med, 1998; 339: 69-75.

65. Perico N, Amuchastegui CS, Colosio V. 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, 1994; 5: 1139-46.

66. Trivedi HS, Pang MMH, Campbell A, Saab P. Slowing the progression of chronic renal failure: economic benefits and patients' perspectives. Am J Kidney Dis, 2002; 39: 721-9.

67. Hall WD. Abnormalities of kidney function as a cause and a consequence of cardiovascular disease. Am J Med Sci, 1999; 317: 176-82.

68. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol, 1999; 10: 1606-15.

69. Mann JFE, Gestein HC, Pogue J, Bosch J, Yusuf S. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of Ramipril: the HOPE randomized trial. Ann Intern Med, 2001; 134: 629-36.

70. Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. Lancet, 2000; 356: 147-52.

71. Sdevanski A, Schmidt KG, Waldherr R, Ritz E. Early increase in blood pressure and diastolic left ventricular malfunction in patients with glomerulonephritis. Kidney Int, 1996; 50: 1321-6.

72. Kronenberg F, Kuen E, Ritz E, Konig P, Kraatz G, Lhotta K, Mann JFE, Muller GA, Neyer U, Riegel W, Riegel P, Schwenger V, von Eckardstein A. Apolipoprotein A-IV serum concentrations are elevated in patients with mild and moderate renal failure. J Am Soc Nephrol, 2000; 11: 105-15.

73. Jungers P, Joly D, Massy Z, Chauveau P, Nguyen AT, Aupetit J, Chadefaux B. Sustained reduction of hyperhomocysteinaemia with folic acid supplementation in predialysis patients. Nephrol Dial Transplant, 1999; 14: 2903-6.

74. Fliser D, Pacini G, Engelleiter R, Kautzky-Willer A, Prager R, Franek E, Ritz E. Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int, 1998; 53: 1343-7.

75. Gall M-A, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving H-H. Albuminuria and poor glycemic control predict mortality in NIDD. Diabetes, 1995; 44: 1303-9.

76. Yusuf S, Ionn E. Anti-ischemic effects of ACE-inhibitors: review of currentclinical evidence and ongoing trials. Eur Heart J, 1998; 19: 36-44.

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, 2003; 14: 178-85.

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, 2000; 342: 145-53.

79. Shlipak MG. Pharmacotherapy for heart failure in patients with renal insufficiency. Ann Intern Med, 2003; 138: 917-24.