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Review

Targeting the renin angiotensin system for remission/regression of chronic kidney disease

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Summary. In the last few years great progress has been made in the search for the cellular and molecular mechanisms of chronic kidney disease and its progression to end-stage renal failure. The possibility of remission/regression of chronic nephropathy has become a reality for some patients on therapy based on renin-angiotensin system blockade – an example of how a public health concern can be successfully addressed by translational medicine. This review describes experimental and clinical investigations documenting the advances achieved in the management of chronic kidney diseases by targeting angiotensin II.

Key words: Angiotensin II, RAS blockade, Chronic kidney disease, Remission, Regression

Introduction

Chronic kidney disease (CKD) is a major cause of morbidity and mortality. Independently of the primary insult, progression to end-stage renal disease (ESRD) is unfortunately the common outcome. The rate of progression varies among nephropathies and for the same disease in different individuals; patients whose CKD inexorably evolves to renal insufficiency are at high risk of dying from cardiovascular complications (Dirks et al., 2005; Perico et al., 2005). CKD that requires renal replacement therapy (RRT) – primarily kidney transplantation, hemodialysis and peritoneal dialysis – is on the increase world-wide and the number of patients estimated to progress toward ESRD may well exceed two million by the year 2010 (Xue et al., 2001). Thus, CKD is emerging as a global threat to human health and represents a major public health challenge for nephrologists in the 21st century.

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Glomerular hypertension, angiotensin II and proteinuria in the progression of chronic nephropathy

Over the last two decades research in experimental models and in humans has focussed on clarifying the mechanism(s) responsible for renal disease progression in an attempt to identify therapeutic targets and therefore to delay or prevent progression to ESRD and the need for RRT. Intraglomerular hemodynamic changes and proteinuria are key determinants of this progression.

Glomerular hypertension and angiotensin II

In the early 1980s Brenner and coworkers introduced the concept that following nephron loss due to the original insult, the remnant nephrons undergo glomerular hypertrophy and rising intraglomerular capillary pressure that leads to hyperfiltration (Hostetter et al., 1981; Brenner et al., 1982). This response, initially compensatory, later becomes maladaptive, contributing to the impairment of the permselectivity of the glomerular barrier and consequent development of proteinuria and progressive glomerulosclerosis (Brenner et al., 1982).

The theoretical model for the size-selectivity function of the glomerular capillary wall (GCW), the heteroporous membrane model, is based on the assumption that the GCW is perforated by hypothetical cylindrical pores with a size distribution (Deen et al., 1985). Small pores dominate in number and serve as the main pathway for water and small molecules across the glomerular membrane, but small population of large pores shows negligible selectivity, even for molecules of molecular radius 60Å, and these form the so-called "shunt" pathway through which 1% of filtrate volume

passes (Deen et al., 1985). Experimental investigation of glomerular permselectivity defects in passive Heymann nephritis, a model of membranous glomerulopathy, in which there is an abnormally high transcapillary hydraulic pressure difference and massive proteinuria, has documented an increase in the ratio of large to small pores, suggesting that proteinuria depends on recruitment of previously unexposed non-selective pores that offer an escape pathway permeable to macromolecules (Yoshioka et al., 1987).

Glomerular hypertension exposes glomerular cells to mechanical load that affects their functions, including differentiation and proliferation, matrix production and intracellular signal transduction. Mesangial cells respond to mechanical stretch *in vitro* by proliferating (Ingram et al., 1999), whereas podocytes undergo actin cytoskeleton reorganization (Endlich et al., 2001), hypertrophy (Petermann et al., 2005), reduced adhesion through alpha 3 beta1 integrin down-regulation (Dessapt et al., 2009), and apoptosis due to local activation of the angiotensin system (Durvasula et al., 2004). Increased angiotensin II (Ang II) production by mechanically stretched podocytes might in its turn raise intraglomerular capillary pressure (Yoshioka et al., 1987), thus setting in motion a vicious circle that perpetuates further damage to the podocyte. Ang II has also a direct effect on podocyte functions (Fig. 1). It depolarizes them by opening up chloride conductance through the angiotensin type 1 receptor (AT1R) (Gloy et al., 1997). The intracellular calcium that is increased by Ang II (Nitschke et al., 2000), quite likely regulates the activation of this ion conductance. Ang II induces cytoskeletal reorganization and shedding of the slit diaphragm-associated protein nephrin, the key regulator of the filtration barrier (Doublier et al., 2003). Ang II-induced reorganization of F-actin fibers is also instrumental for the redistribution of zonula occludens-1 (ZO-1), a functionally important molecule of the foot process that is physically associated with actin in podocytes, and with other actin-related proteins, including α-actinin. This results in permselective dysfunction of podocyte-podocyte contact and increased albumin permeability (Macconi et al., 2006a). Changes in both F-actin and ZO-1 patterns are found in glomeruli of rat isolated perfused kidneys after short infusion of Ang II, leading to increased protein excretion (Macconi et al., 2006a). All these findings indicate Ang II's direct action in perturbing the glomerular sieving function, which is independent of hemodynamic changes. Ang II induces podocyte dysfunction through the AT1R, partially dependent on Src kinase-phospholipase C (PLC) activation (Macconi et al., 2006a).

Another mechanism whereby Ang II causes actin cytoskeleton reorganization has been recently elucidated. Persistent renin-angiotensin system (RAS) activation, such as that in cultured podocytes with stable AT1R expression exposed to Ang II, results in reactive oxygen

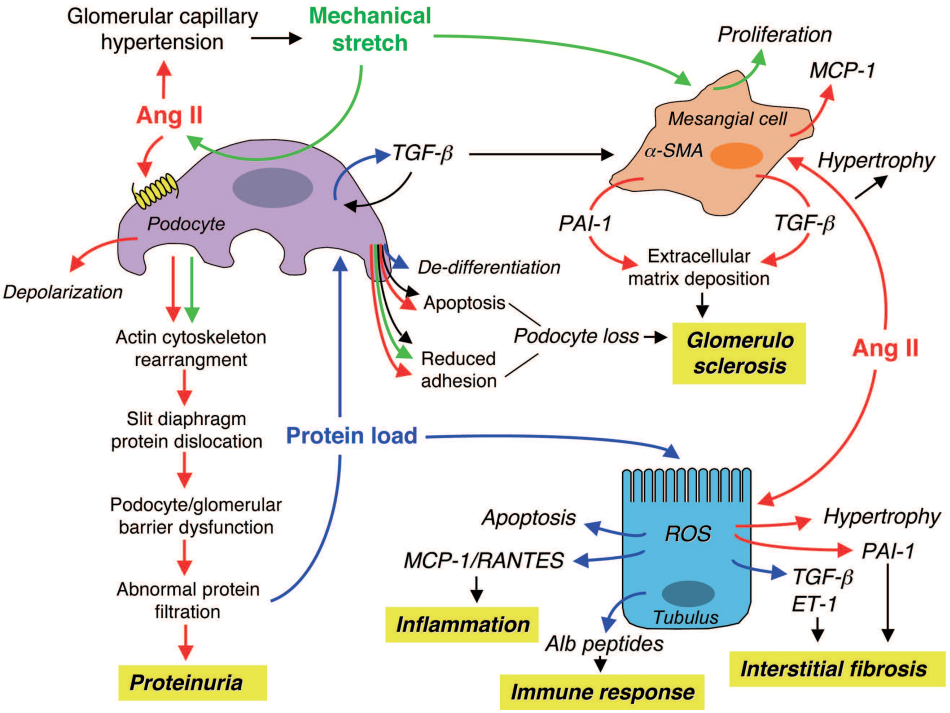

Fig. 1. Interplay between mechanical stretch, Ang II, and protein load in renal disease progression. Glomerular capillary hypertension mechanically stretches mesangial cells and podocytes with two opposite effects: respectively proliferation and distress/apoptosis. The latter is partly due to local activation of Ang II, which in turn raises intraglomerular capillary pressure, thus perpetuating podocyte damage. Activation of Ang II signaling leads to podocyte dysfunction and ultimately to loss of permselectivity of the glomerular barrier. Abnormal filtration of proteins results in proteinuria. Accumulation of proteins (protein load) in the podocytes causes cell de-differentiation and injury and up-regulation of TGF-ß that triggers podocyte apoptosis and mesangial cell activation. Increased extracellular matrix synthesis by TGF-ß and reduced degradation by PAI-1 lead to the accumulation of extracellular matrix which, together with podocyte loss, contributes to the development of glomerulosclerosis. Both Ang II and protein load stimulate tubular cells to release pro-fibrotic and inflammatory mediators. The inflammatory enviroment stimulates renal dendritic cells to become immunogenic and to take up albumin fragments generated by distressed tubules and process them into antigenic peptides, triggering an immune response. Abbreviations: Ang II, angiotensin II; ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor 1; TGF-ß, transforming growth factor-ß. In red, Ang II; green, mechanical stretch; blue, protein load signaling.

species (ROS)-dependent F-actin cytoskeleton reorganization and acquisition of a migratory phenotype (Hsu et al., 2008). Activation of small GTPases Rac1 and RhoA and phosphorylation of the ERM (ezrin/radixin/moesin) proteins gives the intracellular signaling involved in cortical F-actin ring formation and down-regulation of α-actinin 4. This protein regulates the podocyte-matrix interaction and is required for normal podocyte adhesion (Dandapani et al., 2007). Mutation or depletion of α-actinin 4 is linked to focal segmental glomerulosclerosis (Kaplan et al., 2000; Kos et al., 2003). Intracellular signaling downstream Ang II-induced Rac1 activation is probably responsible for podocyte detachment and the development of glomerulosclerosis in transgenic rats overexpressing AT1R specifically and exclusively in podocytes (Hoffmann et al., 2004).

Ang II also contributes to renal disease progression through its pro-fibrotic and pro-inflammatory effects by targeting other resident glomerular cells besides podocytes and tubules (Fig. 1). It stimulates extracellular matrix (ECM) synthesis in mesangial cells by inducing transforming growth factor ß (TGF-ß) (Kagami et al., 1994); it also triggers plasminogen activator inhibitor-1 (PAI-1) production by mesangial and tubular cells, thus favouring ECM deposition in the glomerulus and interstitial space (Wilson et al., 1997; Nakamura et al., 2000; Fintha et al., 2007) and induces mesangial and tubular cell hypertrophy (Anderson et al., 1993; Jaimes et al., 1998; Hannken et al., 1998).

Ang II concurs in the development of glomerular inflammation by activating in mesangial and glomerular endothelial cells nuclear factor-κB (NF-κB)-dependent genes for the chemokines monocyte chemoattractant protein-1 (MCP-1) (Ruiz-Ortega et al., 1998) and RANTES (Wolf et al., 1997) that recruit monocytes/macrophages within the tuft. *In vivo* evidence of Ang II-induced phenotypic changes of glomerular cells associated with glomerular inflammatory cell infiltrates (De Craemer et al., 2001), as well as redox imbalance and increased oxidative stress, upholds the causal role of Ang II in renal damage (Haugen et al., 2000).

Protein load

Rather than simply a marker of damage, abnormal filtration of plasma proteins through the GCW has intrinsic toxicity on the proximal tubule and subsequently on the whole kidney. Proximal tubular cells exposed *in vitro* to high concentrations of albumin undergo apoptosis through a mechanism involving reduced expression of its receptor megalin that binds to protein kinase B (PKB) at the plasma membrane, reducing PKB activity and phosphorylation of Bad protein by PKB (Caruso-Neves et al., 2006). Overload of plasma proteins (albumin, IgG and transferrin) stimulates proximal tubular cells to synthesize and release inflammatory mediators responsible for macrophage, lymphocyte, and neutrophil recruitment (MCP-1, RANTES and interleukin 8) and pro-fibrotic factors such as endothelin-1 (ET-1) and TGF-ß, the most potent inducer of epithelial-to-mesenchymal transition (EMT) (see for reviews Abbate et al., 2006; Strutz, 2009) (Fig. 1). Protein kinase C-dependent hydrogen peroxide production, extracellular signal-regulated kinases and mitogen-activated protein kinases, as well as signal transducer and activator of transcription (STAT) proteins, are intracellular signaling pathways involved in the NF-κB-dependent activation of chemokines induced by protein load, mainly albumin (Abbate et al., 2006). *In vivo* studies have convincingly documented a causal association between proteinuria and tubulo-interstitial disease, correlating with the degree and rate of progression of renal failure. Activation of chemokine and ET-1 pathways has been proved by detection of high mRNA and/or protein levels during interstitial infiltration of inflammatory and immune cells (Abbate et al., 2006). Among the latter, $CD8^+$ T cells and dendritic cells (DC) that accumulate in the renal parenchyma are hallmarks of proteinuric nephropathies even in the absence of an immune insult. Recently a link has been described between the biology of the tubular epithelial cells and the role of DC (Macconi et al., 2009a), the main professional antigen-presenting cell population of the healthy kidney (John and Nelson, 2007). Proteolysis of excess albumin by proximal tubular cells provides the substrate to DC for the generation – through a proteasome-dependent pathway – of antigenic peptides recognized by $CD8^+$ T cells (Macconi et al., 2009a). Normally ignored self-proteins can, on renal injury, generate antigenic peptides, thus triggering an immune response.

The gene expression profile of renal proximal tubules micro-dissected from mouse proteinuric kidneys has revealed dramatic changes in the expression pattern compared to normal animals (Nakajima et al., 2002). Over 1000 genes are upregulated by proteinuria, including those involved in the albumin metabolism and/or degradation pathway, as well as in inflammation and immunity (Nakajima et al., 2002). Similarly, cDNA microarray of proximal tubular cells from patients with proteinuric nephropathies showed more than 160 differentially expressed genes including those involved in signal transduction, cell proliferation and cell cycle control, differentiation, immune response, and intracellular transport and metabolism (Rudnicki et al., 2007).

Besides the proximal tubular cell, the podocyte is the cellular target of protein load. In the renal mass ablation model intraglomerular accumulation of plasma proteins mainly in podocytes is observed one week after surgery and precedes myofibroblast transformation of the surrounding mesangial cells (Abbate et al., 2002). Morphological changes consist of segmental adhesion of the capillary tuft to the Bowman's capsule that develops into glomerulosclerosis. Protein-overloaded podocytes undergo de-differentiation and injury, as evidenced by

loss of synaptopodin expression and increased staining for desmin, with up-regulation of TGF-ß1 expression (Fig. 1). This latter is responsible for sclerosing activation of mesangial cells (Abbate et al., 2002). TGF-ß1 can have autocrine effects on podocytes, inducing EMT (Li et al., 2008), reduced adhesion (Dessapt et al., 2009), and apoptosis (Schiffer et al., 2001; Niranjan et al., 2008) which lead to podocyte dysfunction and loss (Fig. 1).

These findings all uphold the concept that excess proteins are *per se* toxic on glomerular and tubular cells and make their own contribution to renal disease progression independently of Ang II. This is further borne out by evidence that targeted deletion of AT1a does not protect mice from progressive nephropathy of overload proteinuria (Benigni et al., 2004).

From retarding renal disease progression to remission of CKD: renoprotection by RAS blockade

Proteinuria is a reliable biomarker of the severity of renal disease and predicts the risk of progression. Changes in proteinuria and in the decline in glomerular filtration rate (GFR) are closely correlated in diabetic (Breyer et al., 1996) and non-diabetic renal disease (Peterson et al., 1995; Ruggenenti et al., 1998b). A study in a large Caucasian population reported that proteinuria independently predicted the risk of ESRD and overall mortality (Tarver-Carr et al., 2000). Mass-screening of more than 100,000 individuals in Japan found, during a 17-year follow-up, a positive relationship between baseline proteinuria (dipstick urine analysis) and the risk of developing ESRD (Iseki et al., 2003). Even a slight increase in proteinuria was an independent risk factor for ESRD. Evidence that reducing proteinuria always results in a better disease outcome further supports its pathogenic role in the progression of CKD. The Modification of Diet in Renal Disease study showed that reduction of proteinuria was associated with a decrease in the rate of GFR decline, and that protection achieved by lowering blood pressure depended on the extent of initial proteinuria (Peterson et al., 1995).

Angiotensin-converting enzyme inhibitors in non-diabetic nephropathies

In the Ramipril Efficacy in Nephropathy (REIN) trial (Core study), patients with non-diabetic proteinuric chronic nephropathy, after stratification for baseline proteinuria 1-3 g/day (stratum 1) or ≥ 3 g/day (stratum 2), were randomly assigned to receive the angiotensin-converting enzyme inhibitor (ACEi) ramipril or placebo, plus conventional antihypertensive therapy to maintain diastolic blood pressure at 90 mmHg or less (Gisen Group, 1997). The effects of both treatments on the following end points were compared: proteinuria, GFR decline, and ESRD. The GFR declined faster in patients with higher urinary protein excretion at baseline (≥ 3 g/day). These were the patients who benefited most from ACE inhibition. Despite comparable blood pressure control, ramipril was more renoprotective than placebo in these patients; it significantly slowed renal function decline and halved the combined risk of doubling of serum creatinine or end-stage renal failure – ESRF (Gisen Group, 1997). Proteinuria significantly decreased by month 1 in the ramipril-treated patients and remained lower than baseline throughout the study, whereas it did not change in the placebo group. In the ramipril group, the one-month proteinuria reduction was also inversely correlated with long-term rate of GFR decline (six months or more after randomization) (Gisen Group, 1997).

This study provides the first demonstration that the beneficial effects of ACEi on renal function deterioration go beyond the anti-hypertensive action. A post-hoc analysis of the REIN study data indicated that besides basal proteinuria, residual proteinuria predicts progression of CKD to ESRF independently of blood pressure control and treatment randomization (Ruggenenti et al., 2003).

Meta-analysis of data from 1860 patients, enrolled in 11 randomized controlled trials (including the REIN study), comparing the effects of antihypertensive regimens, including ACEi or not including ACEi, on the progression of non-diabetic renal disease, confirms and extends previous findings. A higher level of proteinuria, either at baseline or during the follow-up, is an independent risk factor for the progression of non-diabetic renal disease (Jafar et al., 2001). The greater renoprotective effects of ACEi in patients with higher baseline urine protein excretion reflect their greater antiproteinuric effect in these patients.

In view of the efficacy of ramipril, the REIN Core study was prematurely stopped and all patients with proteinuria (≥ 3 g/day) were put on ramipril therapy regardless of the original randomization and followed for two years (REIN follow-up). This study showed that prolonged ACE inhibition efficiently stopped the tendency of GFR to decline with time and prevented the risk of progression to ESRF (Ruggenenti et al., 1998a). After 36 months of continued ramipril treatment, no patient progressed to the point of requiring dialysis, whereas 30% of patients originally randomised to placebo plus conventional antihypertensive therapy and switched to ramipril still developed ESRD (Ruggenenti et al., 1998a).

These data suggest that the earlier the ACEi treatment starts the greater the effect in protecting from GFR decline and ESRD. In patients who continued on ramipril, with at least six GFR determinations, the mean rate of GFR decline improved with time, parallel with a significant reduction in proteinuria, and in the cohort with the longest follow-up (60 months) it reached about 1 mL/min per 1.73 m^2 per year, approximating the physiologic age-related loss of GFR with time in people with no evidence of renal disease. Analyses of the slopes in individual patients confirmed stabilization or even an increase in GFR in some cases (Ruggenenti et al., 1999).

Thus, remission or even regression of non-diabetic chronic nephropathy can be achieved in some patients by prolonged ACEi therapy.

ACEi in diabetic nephropathies

In a prospective study in a small number of insulin-dependent diabetic patients long-term antihypertensive treatment with an ACEi (≥ 6 years) progressively reduced the rate of GFR decline (Parving et al., 1987). In another study, at comparable blood pressure, the ACEi were more effective than a beta blocker in reducing proteinuria and the rate of decline of kidney function (Bjorck et al., 1992). In a large randomized controlled trial in 409 type 1 diabetic patients the ACEi captopril approximately halved the risk of the combined end-points of doubling serum creatinine, ESRD or death, as compared to placebo (Lewis et al., 1993). Some patients who had nephrotic syndrome (proteinuria ≥ 3.5 g/day), randomized to ACEi enjoyed long-term remission of the nephrotic syndrome (proteinuria ≤ 1g/day) and stabilization of GFR during an eight-year follow-up (Wilmer et al., 1999).

A multimodal approach in CKD patients

A multimodal approach was formalized in an intervention protocol, "the Remission Clinic program" for patients with CKD and heavy proteinuria despite ACEi therapy (Ruggenenti et al., 2001b). Evaluation of the rate of GFR decline and incidence of ESRD in a cohort of 56 patients with nephrotic range proteinuria (> 3 g/day) enrolled in this program showed that over a median follow-up of four years multidrug treatment titrated to the urinary protein level slowed GFR decline and reduced the risk of ESRD 8.5-fold. Normalization of proteinuria and stabilization of GFR was achieved in 26 patients who would have otherwise been expected to progress rapidly to ESRD on conventional therapy titrated to target blood pressure (Ruggenenti et al., 2008).

How blockade of the RAS provides renoprotection

Experimental models of progressive nephropathies have provided more information on the mechanisms through which inhibition of Ang II can achieve renoprotection. The sieving properties of the glomerular membrane by Ficoll fractional clearance in a model of spontaneous glomerular injury and in diabetes indicated that both ACEi and angiotensin receptor blockers (ARBs) prevent proteinuria by preserving the size-selective function of the glomerular capillary (Remuzzi et al., 1990, 1993). This has also been demonstrated in human renal diseases such as diabetic, IgA, and membranous nephropathies (Parving et al., 1988; Morelli et al., 1990; Remuzzi et al., 1991; Ruggenenti et al., 2000). Preservation of the permselective properties of the glomerular capillary reflects the fact that Ang II blockade prevents the dislocation and/or loss of slit diaphragm-associated molecules (i.e. ZO-1 and nephrin), which are essential for maintaining the filtration barrier (Macconi et al., 2000; Benigni et al., 2001b; Bonnet et al., 2001; Kelly et al., 2002). Targeting proteinuria by Ang II blockade provides renoprotection by preventing glomerulosclerosis, glomerular-tubule disconnection and atrophy and interstitial inflammation (Remuzzi et al., 1994; Abbate et al., 1999; Benigni et al., 2001a).

The podocyte is a therapeutic target of RAS blockade; preservation of its structural and functional integrity is instrumental to nephron function. Podocyte dysfunction plays a key role in proteinuria and glomerulosclerosis (see for reviews Shankland, 2006; Wiggins, 2007). Podocyte loss is a causal factor for renal disease progression in animals (Kim et al., 2001; Kuhlmann et al., 2004; Wharram et al., 2005; Wiggins et al., 2005; Macconi et al., 2006b) and humans (Pagtalunan et al., 1997; Meyer et al., 1999; Steffes et al., 2001; White et al., 2002; Lemley et al., 2002; Dalla Vestra et al., 2003). Podocyte detachment from the glomerular basement membrane and loss into the urine is a cause of podocytopenia (Vogelmann et al., 2003; Petermann et al., 2003). Urinary podocytes may be a useful marker of disease activity in experimental models (Yu et al., 2005) and in patients with diabetic (Nakamura et al., 2000b) or IgA nephropathy (Nakamura et al., 2000c). They have also been suggested as a diagnostic indicator for differentiating focal segmental glomerulosclerosis and minimal change nephrotic syndrome (Nakamura et al., 2000a). Both ACEi and ARBs prevent podocyte loss in experimental diabetes (Gross et al., 2003a,b) and podocyturia in human nephropathies (Nakamura et al., 2000b,c).

Long-term treatment of MWF rats, uni-nephrectomized to accelerate renal disease progression, with ACEi that completely prevents proteinuria, also prevents ESRF and improves survival (Remuzzi et al., 1995). Like in animals, long–term remission of nephrotic-range proteinuria achieved by treatment with ACEi or ARBs can also reduce the risk of ESRD and all-cause mortality in patients with type 2 diabetes (Rossing et al., 2005).

Regression of glomerulosclerosis and glomerular cell remodeling: information from experimental models

Studies in animals and in patients with type 1 diabetes with sustained normoglycemia after pancreatic islet or pancreas transplantation have proved that regression of glomerulosclerosis is possible (Mauer et al., 1975; Fioretto et al., 1998). But can drug therapy reverse glomerular lesions? In experimental models of progressive nephropathies treatments based on Ang II blockade started in the early stages of the disease help prevent renal disease progression (see above). In MWF rats, a genetic model of progressive nephropathy, ACEi given when proteinuria is already important still exert

renoprotection by reducing the urinary protein excretion rate below baseline and halting the progression of renal damage (Remuzzi et al., 1999).

Lately scientists' attention has moved to more advanced stages of the disease with the purpose of verifying the efficacy of treatment once glomerular lesions are already manifest. This mimics therapeutic intervention in patients who show signs of renal failure when they come to the physician's attention. Fogo's group was the first to investigate the efficacy of a delayed treatment with different ACEi dosages on established glomerular sclerosis in a model of subtotal nephrectomy (Ikoma et al., 1991). Glomerular sclerosis was assessed on glomeruli sampled from serially sectioned kidney specimens at biopsy and at autopsy, and were ranked on the basis of their severity. In untreated animals sclerosis advanced during the four weeks after biopsy in all glomeruli, while low-dose enalapril attenuated the progression of sclerosis, preserving the structure of glomeruli with early or no sclerotic lesions. However, glomeruli with more advanced sclerosis at biopsy, which tend to progress further, did not benefit from the treatment. Compared to the low dose, high-dose ACEi, was equipotent in controlling both systemic and glomerular hypertension, but had greater beneficial effects on glomerular structure (sclerosis being less at autopsy than at biopsy in half the treated rats).

The same group extended these observations to other experimental models. Six months' treatment of normotensive aging rats with the ARB, losartan, induced regression of vascular and glomerular lesions, with a significant reduction of kidney collagen content. Proteinuria was also reduced and apoptosis of tubular and interstitial cells was less than at baseline. AT_1R blockade attenuated TGF-ß1 mRNA and protein expression and markedly reversed PAI-1 mRNA and protein expression (Ma et al., 2000).

Control of extracellular matrix accumulation by inhibiting PAI-1 or TGF-ß1 has also been proposed as underlying the therapeutic effect of Ang II blockade respectively in the 5/6 nephrectomy (Ma et al., 2005) or nitric oxide (NO) deficiency model (Boffa et al., 2003). In both studies, regression of glomerulosclerosis was independent of the ARBs' effects on the expression of matrix metalloproteases MMP-2 and MMP-9, which play a key role in glomerular collagen remodeling. In rats with 5/6 nephrectomy that achieved regression of sclerosis in response to treatment, the lower PAI-1 expression was paralleled by restoration of plasmin activity in the kidney, indicating degradation of extracellular matrix proteins as a key process to remodeling of sclerosis. Activation of MMPs observed in the NO model, probably due to an adaptive response to kidney fibrosis, additionally contributed to sclerosis reabsorption in losartan-treated rats (Boffa et al., 2003).

In the 5/6 nephrectomy model, exuberant RAS activation, due to the high expression of AT1R within the inflamed renal parenchyma, might hamper complete renoprotection by ACEi or ARBs at "conventional" antihypertensive doses (Goncalves et al., 2004). An extremely high dose of the ARB losartan was therefore used in an attempt to obtain complete renoprotection. With a comparable antihypertensive effect, an ultrahigh dose of losartan did indeed provide maximal protection by halting renal inflammation and glomerular/interstitial damage at pre-treatment levels, and promoted the regression of urinary albumin excretion (Fujihara et al., 2005).

Ultrahigh-dose ARB candesartan also showed superior protection against chronic renal inflammation in spontaneously hypertensive rats, attributed to its particular antioxidant action unrelated to AT1R blockade (Chen et al., 2008). The effect of delayed treatment with ultrahigh-dose losartan compared to a conventional dose on the extent of moderate or advanced glomerular lesions was also studied in type 1 diabetic rats maintained in a state of moderate hyperglycemia by daily insulin injections for ten months (Teles et al., 2009). Independently of the dose, losartan induced the regression of both albuminuria and glomerular injury, as documented by a reduction of mesangial expansion and of the severity of sclerotic lesions to below baseline. However, the percentage of glomeruli with severe sclerotic lesions associated with synechiae to Bowman's capsule was similar to that before treatment, suggesting incomplete resolution of advanced glomerulosclerosis (Teles et al., 2009).

Further investigation of the volume of sclerosis and remodeling of the glomerular structure with treatment is now possible with three-dimensional reconstruction of the entire capillary tuft. This technique was instrumental in demonstrating that in MWF rats with very advanced nephropathy a high dose of ACEi not only markedly reduced sclerosis volume in most glomeruli, but also increased the volume of intact capillaries by up to 40%, indicating substantial glomerular tuft repair (Remuzzi et al., 2006). Regression of glomerular lesions was associated with regression of proteinuria and stabilization of renal function. Worsening of interstitial changes was also prevented.

These findings raised the question whether glomerular tuft repair is due to restucturing or regeneration, and which glomerular cell component is involved (Joles et al., 2006). In subtotally nephrectomized rats, reversal of glomerulosclerosis by high-dose ACEi was associated with a reduction in glomerular volume due to the decreases in mesangial and endothelial cells per glomerulus and in capillary number (Adamczak et al., 2003, 2004). These data suggest that reversal of glomerular lesions involves restructuring of the glomerular microvasculature. In a different variant of the remnant kidney model, however, reabsorption of glomerulosclerosis with ARB treatment was paralleled by increased complexity and branching of capillaries, suggesting potential regeneration of glomerular segments (Fogo, 2005; Scruggs et al., 2005).

In MWF rats, in which loss of podocytes with age

contributes to disease progression (Macconi et al., 2006b), regression of glomerular lesions in response to ACEi was associated with remodeling of glomerular cell components, resulting in a selective increase in podocyte number from baseline (Macconi et al., 2009b). Besides podocyte repopulation, enhanced endothelial cell volume density and reduced mesangial hyperplasia were observed in response to treatment (Macconi et al., 2009b). That the increases in podocytes might be the driving event for endothelial cell remodeling in response to ACEi is supported by recent findings that ARB can restore podocyte potential to promote glomerular endothelial cell sprouting, proliferation, and migration through the induction of podocyte-derived angiogenic factors (Liang et al., 2006).

The mechanism underlying the increase in podocyte number per glomerulus induced by ACE inhibition is still not clear. Repopulation of podocytes in the glomerular tuft was associated with an increase in the number of cells positive for both the podocyte-specific nuclear antigen WT1 and the proliferation marker Ki-67 (Macconi et al., 2009b). Considering that podocytes have limited ability to proliferate, these positive cells might derive from podocyte precursors. Ultrastructural features characteristic of podocytes (i.e. foot processes) have been documented in some parietal epithelial cells (PECs) of the Bowman's capsule (Gibson et al., 1992; Macconi et al., 2009b). These cells express epitopes of mature visceral podocytes and retain the ability to divide (Bariety et al., 2006); "transitional cells" – so called because of their intermediate phenotype between PECs and podocytes –, are mainly located in the glomerular vascular stalk (Appel et al., 2009).

In MWF rats the parietal podocytes as a percentage of total PECs increased in response to ACE inhibition (Macconi et al., 2009b). Electron microscopy revealed continuity between the inner surface of the Bowman's capsule and the outer glomerular capillary membrane, indicating that parietal cells can probably migrate from Bowman's capsule to the capillary tuft even in normal physiological conditions (Macconi et al., 2009b) (Fig. 2). The occasional finding of PECs protruding toward the tuft suggests that these cells can also migrate to the tuft through cellular bridges in areas other than the vascular pole (Macconi et al., 2009b).

Overall, these results suggest that remodeling of the PECs of the Bowman's capsule contributes to podocyte restoration in response to ACE inhibition. This is further supported by two recent studies showing that PECs do regenerate podocytes. Monitoring the fate of genetically tagged PECs and their progeny in juvenile transgenic mice provided experimental proof that PECs are recruited onto the glomerular tuft and differentiate into podocytes (Appel et al., 2009). These findings parallel the identification in the adult human kidney of a subset of renal progenitor cells that express the stem cell markers $CD133^+CD24^+$ but no podocyte markers (podocalixin, PDX^-), exhibit self-renewal potential and high cloning efficiency, and act as bipotent progenitors for both tubules and podocytes (Sagrinati et al., 2006; Ronconi et al., 2009). These cells are localized at the urinary pole of the Bowman's capsule and are distinct from a transition population expressing both renal progenitors and podocyte markers that is present between the urinary and the vascular poles, and can differentiate only into podocytes. Injection of $CD133^+CD24^+PDX^-$ cells into adriamycin-treated Severe Combined Immunodeficiency (SCID) mice, a model of progressive nephropathy involving podocyte depletion, results in these cells being grafted into both glomerular and tubular structures. In the glomerulus, progenitor cells acquire podocyte-specific markers, regenerating podocytes (Ronconi et al., 2009).

All the above evidence suggests that regression of the glomerular scarring induced by ACEi implies a pleiotropic effect not simply based on the control of extracellular matrix deposition, but also on the remodeling of resident glomerular cells and very likely of renal progenitors too (Fig. 3).

Dual RAS blockade and multidrug approach

Although ACEi and ARB have comparable beneficial effects on progressive renal diseases in experimental models, the combination has been tested as a way to maximize RAS blockade and improve renoprotection. In MWF rats with overt nephropathy combined treatment with lisinopril and valsartan normalized proteinuria, halted progressive glomerulosclerosis, and reversed type III collagen accumulation and protein casts. Treatment also suppressed inflammatory cell infiltrates in the renal parenchyma (Remuzzi et al., 2002). Multidrug intervention based on dual blockade of RAS with the addition of statins induced the regression of proteinuria, stabilization of renal function, and complete renoprotection of glomerular and tubular morphology in a severe model of progressive nephropathy resistant to ACEi alone (Zoja et al., 2002).

In experimental studies high-dose ACEi and high/ultrahigh-dose ARB provide greater renoprotection than conventional doses. On clinical grounds, ultrahigh doses of the ARB candesartan had a greater antiproteinuric effect than a standard dose in patients with CKD and overt proteinuria (Schmieder et al., 2005). However, whether the antiproteinuric effect translates into a slower rate of renal and cardiovascular endpoints was not addressed in that study. Type 2 diabetic patients with hypertension and micro or macro albuminuria randomized to higher doses of the ARB valsartan showed a two-fold reduction in urinary albumin excretion rate as compared with patients given the doses commonly used for blood pressure control. Twice as many patients on the highest dose returned to normal albuminuria (Hollenberg et al., 2007). However, additional renoprotection in terms of greater reduction of proteinuria, GFR decline, and rate of doubling serum creatinine, ESRD or death is not peculiar to high-dose

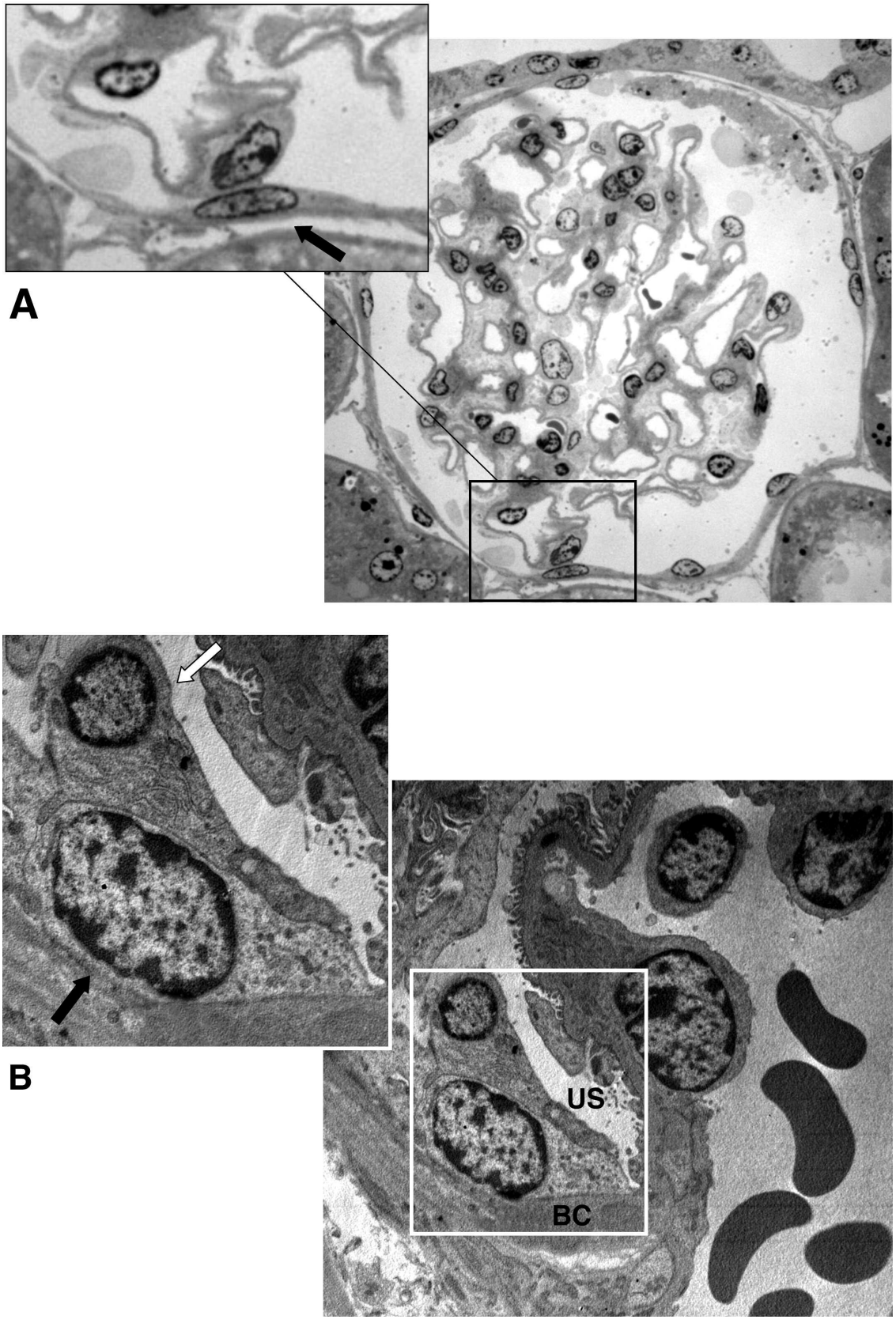

Fig. 2. Connection between parietal epithelial cells and visceral podocytes. **A.** Semithin section of a glomerular tuft. Arrow indicates a parietal epithelial cell (PEC) connecting Bowman's capsule with the glomerular capillary tuft. **B.** Representative transmission electron microscopy image of the glomerular vascular pole showing the connection between a visceral podocyte (white arrow) and a PEC (black arrow). BC, Bowman's capsule; US, urinary space.

ARBs, as it is equally achieved by ACEi up-titrated to the maximal tolerated dose (Hou et al., 2007).

Combined therapy with ACEi and ARB has been used in CKD patients to minimize proteinuria and optimize renoprotection. Except in one study (Agarwal, 2001), dual RAS blockade had a greater antiproteinuric effect than monotherapy (Kunz et al., 2008; Catapano et al., 2008). ARB added on top of ACE inhibition over three years' follow-up was more effective than ACEi alone in slowing the progression of renal insufficiency in hypertensive patients with non-diabetic renal disease, through reduction of proteinuria (Kanno et al., 2006). A recent clinical trial, the ONTARGET study (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) in patients with established atherosclerotic vascular disease or diabetes with end-organ damage, confirmed the efficacy of combined therapy in reducing proteinuria more than monotheraphy. The incidence of ESRD was identical on dual RAS blockade and ACEi or ARB monotherapy (Mann et al., 2008) and this lack of any appreciable additional benefit can be explained on the basis that the rate of GFR decline in ONTARGET patients was within the physiologic range of GFR loss found in adults with aging; this is why the small percentage of those who progressed to ESRD was similar to that observed in the general population, or in patients with diabetes or hypertension, but without proteinuria. Indeed only 4% of ONTARGET patients had overt proteinuria (Ruggenenti and Remuzzi, 2009). Mortality and doubling of serum creatinine were not significantly different with combined therapy or monotherapies. An adverse side effect more frequent in patients on combined therapy – not to be taken for a renal outcome – was the need for acute hemodialysis to treat transient functional impairment. This was quite likely due to transient kidney hypoperfusion (Ruggenenti and Remuzzi, 2009), and recovered when treatment was withdrawn (Mann et al., 2008).

Drugs added on top of dual RAS blockade provide further renoprotection, as documented in the Remission Clinic (see above). Experimental data suggest that multimodal strategy can achieve regression of CKD in patients who do not respond fully to ACEi therapy. This is proved by the case of a young girl with nephrotic proteinuria and systemic lupus who received, in addition to a sodium and protein-restricted diet, triple therapy with an ACEi, an ARB, and a statin. This treatment not only induced remission of proteinuria within six months, but reduction of proteinuria to < 0.3 g/day and improved GFR seven years later indicated regression of CKD (Ruggenenti et al., 2001a).

Conclusions

Regression of proteinuria and remission/regression of glomerular lesions can be achieved by treatment based on RAS blockade in non-diabetic and diabetic experimental nephropathies. Time of administration is important, earlier and more prolonged treatment being more protective. Monotherapy with high or ultra-high doses of ACEi or an ARB can preserve renal structure more effectively than a conventional dose, and a multidrug approach can be useful in case of resistance to Ang II blockade. Translation of experimental knowledge into clinical practice has improved the quality of life for

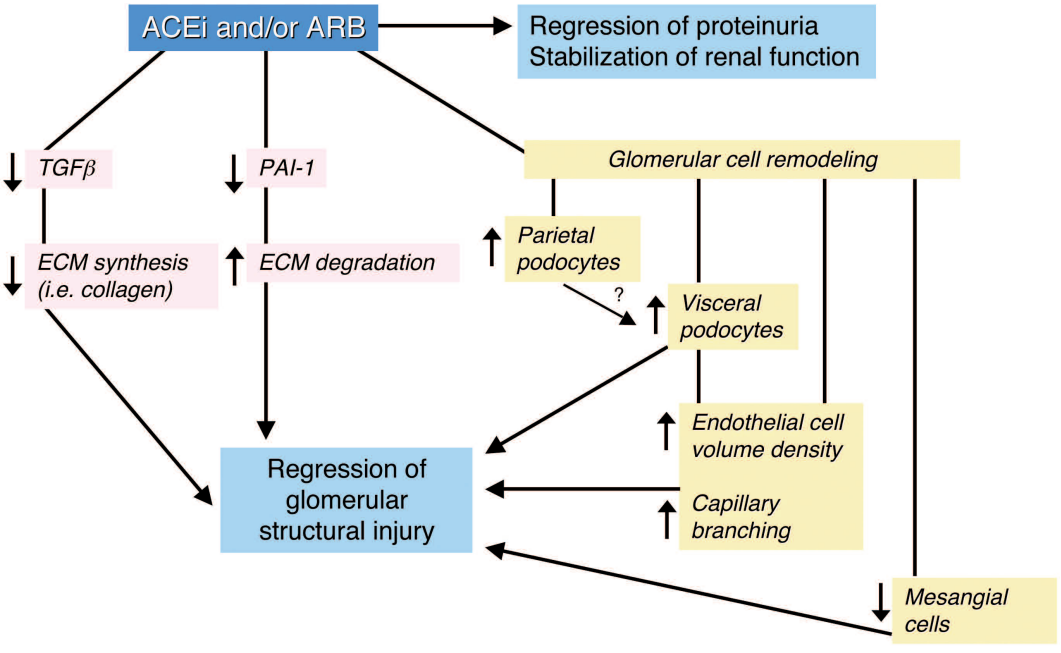

Fig. 3. The mechanisms underlying glomerular capillary restructuring and repair by renin-angiotensin system (RAS) blockade. Control of extracellular matrix deposition and remodeling of glomerular cells are the basis for regression of glomerular structural injury.

patients with CKD and, in some of them – especially those with non-diabetic nephropathies – induced regression. However, despite these positive results, investigations in animal models show that renoprotection is not complete, advanced structural injury such as interstitial inflammation is less prone to regression, and renal function, although stable, does not improve. Similarly, not all patients with CKD benefit from therapy based on RAS blockade. Thus, together with deeper investigation of the mechanisms regulating glomerular cell survival and repair on therapy, research needs to focus on improving knowledge of new pathways not involving Ang II (see review, Perico et al., 2008). Help from tools such as proteomics will help identify new targets and develop novel strategies for patients who remain at high risk for poor renal and cardiovascular outcomes.

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