Advancements in renal protection
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GENERAL INTRODUCTION
Prevention of chronic progressive renal function loss

Chronic kidney disease (CKD) is a worldwide public health problem with a rising incidence and prevalence.¹ In the Netherlands the absolute numbers of patients with end-stage renal disease (ESRD; stage 5 CKD) increased from 3064 to 5259 in the period 1990-2005. In the same period the incidence increased from 1033 new patients with ESRD per year to 1686 (data from the Dutch Renal Replacement Registry; www.renine.nl). Patients with ESRD consume a relatively high part of the health care resources. Despite the magnitude of the committed resources and the substantial improvements in the quality of dialysis therapy such as nocturnal hemodialysis at home, these patients still have an increased mortality and morbidity risk and a reduced quality of life compared to the general population.²;³ Therefore, it is important to slow down or, even better, halt or reverse the progression of renal function loss in CKD patients to prevent ESRD.

Once patients reach ESRD, kidney transplantation is the preferred option for renal replacement therapy, resulting in improved quality of life and reduced mortality compared to dialysis.⁴ Over the last decades, the introduction of new immunosuppressive drugs has markedly reduced the incidence of acute rejection after renal transplantation.⁵;⁶ Short-term graft survival has therefore increased, but unfortunately, no improvements have been obtained regarding long term chronic transplant dysfunction.⁷ Consequently, the main cause of graft loss is chronic allograft nephropathy (CAN). Thus, for both native (CKD) and transplanted kidney damage (CAN), the main challenge is currently the prevention of chronic progressive renal function loss.

CKD and CAN share several common mechanisms of progressive renal damage and consequent renal function loss. These include systemic and intraglomerular hypertension⁸;⁹ as well as proteinuria. Proteinuria is a main risk factor for renal function loss in both native and transplanted kidneys.¹⁰ Whereas formerly it was considered merely a marker for renal damage, it has become increasingly clear that proteinuria has an independent contribution to progressive renal damage. The tubular protein load appears to contribute to tubulo-interstitial inflammation and damage¹¹-¹⁵ and progressive nephron loss. The severity of these tubulo-interstitial lesions predicts the subsequent decline in renal function.¹⁶;¹⁷ Together, the aforementioned factors (hypertension, glomerular hyperfiltration, proteinuria) contribute to a vicious circle of progressive renal function loss in which primary glomerular injury causes proteinuria which further aggravates interstitial damage.
with ultimately loss of nephrons. This is assumed to lead to a compensatory increase of intraglomerular pressure in the remaining nephrons, promoting further glomerular damage, proteinuria and progressive nephron loss. Moreover, proteinuria induces systemic alterations, that may be relevant not only to renal outcome but also to the cardiovascular complications of renal disease. These include sodium retention and edema, dyslipidaemia, hypoalbuminemia and coagulation abnormalities. Likely, these factors contribute to the adverse prognostic impact of proteinuria on renal and cardiovascular prognosis.

**Aim of the thesis**

Clearly, the vicious circle of progressive renal damage has to be broken to prevent progressive renal function loss in CKD and CAN. Functional blockade of the renin-angiotensin-aldosterone system (RAAS), by ACE-inhibitors or AT1 receptor blockade has afforded great progress in renoprotection, by reducing blood pressure, proteinuria and the rate of renal function loss, in particular in proteinuric native kidney disease. Consequently, currently RAAS blockade stands out as the most effective renoprotective treatment. However, as many patients still suffer ongoing renal function loss apparently the renoprotective effect of the current RAAS blockade based regimens remains incomplete. This prompts for optimization of RAAS blockade based therapy to provide better renoprotection (part one of this thesis). Furthermore, additional modes of intervention that directly target other pathophysiological pathways involved in CKD and CAN, might be useful. In part two of this thesis we investigate whether intervention in advanced glycation end products (AGEs) can exert renoprotective effects.

**Renin-angiotensin aldosterone system (RAAS)**

Research on the RAAS, starting with the discovery of renin in 1898, has a history of more than a century already. A schematic and simplified representation of the RAAS is given in Figure 1. Classically, the RAAS has been known as an endocrine cascade that is the main system for regulation of blood pressure, renal hemodynamics and extracellular fluid volume, providing the main line of defense against the circulatory consequences of extracellular volume loss.
Several decades of research support the key role of the RAAS in the homeostatic response to changes in volume status. Briefly, the RAAS is activated by volume depletion (as detected by a decrease in renal perfusion pressure and a low sodium/chloride delivery to the macula densa) and by sympathetic activation. Low perfusion pressure activates baroreceptors in the vascular part of the juxtaglomerular apparatus in the kidneys which stimulates these cells to release the enzyme renin. Renin cleaves the inactive peptide angiotensinogen, converting it into angiotensin I. Angiotensin-converting enzyme (ACE), located mainly in the endothelium of the pulmonary capillaries, then converts angiotensin I to angiotensin II. Angiotensin II induces aldosterone release from the adrenal cortex, leading to distal tubular sodium retention, thus contributing to correction of the volume depletion that initiated the RAAS-activation. Angiotensin II, the primary mediator of the RAAS, acts mainly via the angiotensin II type 1 (AT1) receptor inducing systemic as well as renal vasoconstriction, thus ensuring preservation of systemic and renal perfusion pressure during conditions of volume depletion. Moreover angiotensin II increases proximal tubular sodium reabsorption. Intervention in these functions of the RAAS can thus be assumed to underly...
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the well-established effects of RAAS blockade, i.e. reduction of blood pressure and glomerular pressure, and consequently reduction of proteinuria.

Over the last decade however it has become increasingly clear that the RAAS is not only involved in volume homeostasis, but also in various processes of tissue remodelling in the kidney and the cardiovascular system. By AT1 receptor binding, angiotensin II stimulates cell proliferation and hypertrophy and triggers pro-inflammatory and pro-fibrotic processes. These effects may contribute to progressive renal damage, and likely, interference with these effects is relevant to the renoprotective properties of RAAS blockade as well. Indeed, the time course of onset of antiproteinuric effect of RAAS blockade suggests that only part of the antiproteinuric effect is due to hemodynamic effects, and that a more gradual improvement of glomerular structural integrity is involved as well. In proteinuric rats, for instance, it has been shown that the antiproteinuric effect of ACEi is associated with preservation of glomerular heparan sulphate proteoglycans (HSPGs) thus ameliorating structure of the glomerular basement membrane and its abnormal permeability to proteins. Moreover, not only angiotensin II may be involved in the effects of the RAAS on tissue remodelling. Recent studies suggest that through their pro-fibrotic actions, aldosterone and renin can directly contribute to processes of tissue remodelling and progressive renal damage. Thus, specific blockade of these components of the RAAS may offer prospectives for renoprotection as well.

The role of the RAAS in the diverse processes of tissue remodelling is highly complex, and involves interaction with multiple other pathways. The relevance of such interactions for the pathophysiology of progressive renal damage, or, the other way round, for the renoprotective efficacy of RAAS blockade is still largely unexplored. As mentioned above, data in experimental models suggest effects of RAAS blockade on glomerular HSPGs, and more recently, this was suggested to be due to effects of the RAAS on heparanase (an heparan sulfate degrading enzyme) an observation which prompts for further exploration. Renal accumulation of AGEs may also be relevant in this respect, as discussed in more detail below.

**Improvement of RAAS intervention for optimization of renoprotective therapy**

Intervention in the RAAS with ACE inhibitors and AT1 receptor blockers provides an
important tool to slow the progression of chronic kidney disease.\textsuperscript{42-44} These drugs decrease blood pressure and disproportionately intraglomerular pressure and thereby proteinuria,\textsuperscript{45-46} all important factors of progressive renal function loss in CKD and CAN. However, optimization of RAAS blockade based therapy is needed, since many patients still suffer ongoing renal function loss despite RAAS blockade.\textsuperscript{27,28} Table 1 provides an overview of measures to optimize outcome of RAAS blockade-based therapy.

| Table 1. Strategies for optimization of RAAS blockade based therapy in renal disease |
|-----------------|------------------|
| Increasing dose | Increasing dose |
| Addition of a low protein diet | 18,47,48,50,51 |
| Co-treatment with indomethacin | 52 |
| Dual blockade (combining ACEi and AT1 receptor blockade) | 46,47 |
| Addition of intervention in sodium / volume status: | Addition of intervention in sodium / volume status: |
| Low sodium diet | 18,54,55 |
| Diuretic therapy | 54,56,57 |
| Combination of both | Combination of both |
| Addition of aldosterone receptor blockade | Addition of aldosterone receptor blockade |
| Addition of renin inhibition / blockade | Chapter 3 & 4 |
| Co-treatment with lipid lowering drugs | Chapter 3 & 4 |
| Better monitoring tools of therapeutic efficacy to titrate renoprotective treatment | Chapter 2 |

Increasing the dose of ACEi and/or AT1 receptor blockers is an important first step in the optimization of RAAS blockade.\textsuperscript{18,47} This leads to a further decrease in blood pressure and proteinuria. Of note, the top of the dose-response curve for proteinuria reduction appears to be higher than for blood pressure reduction.\textsuperscript{48-50} Indeed, in type 2 diabetic patients very high doses of the AT1 receptor blocker valsartan reduce albuminuria more than the commonly used dose, an effect that appears to be independent of blood pressure.\textsuperscript{51}

Both dietary protein restriction\textsuperscript{52} and the addition of indomethacin\textsuperscript{53} to ACEi therapy selectively enhance its antiproteinuric effect without a further reduction in blood pressure. Another important optimization strategy, dual RAAS blockade, combining ACEi and AT1 receptor blockade, has better renoprotective efficacy in non-diabetic proteinuric patients compared to either maximum treatment alone.\textsuperscript{46} Moreover, this dual blockade improves long term renal outcome in terms of hard end points.\textsuperscript{46}
Sodium status is an important determinant of the responses of blood pressure and proteinuria to intervention in the RAAS.\textsuperscript{18} ACEi and AT1 receptor blockers are largely ineffective during states of volume excess, either due to renal dysfunction, nephrotic syndrome or to increased sodium intake. Correction of volume overload, or induction of mild volume depletion by dietary sodium restriction or diuretic treatment increases the therapeutic efficacy of RAAS blockade.\textsuperscript{18,54-57} It has been known for more than two decades that intervention in volume status by either low sodium diet, or diuretic therapy, improves the efficacy of RAAS blockade. Surprisingly, the combined effect of low sodium diet and diuretics has not been established. We therefore investigated whether combined intervention in volume status with sodium restriction and diuretic treatment further increased the antiproteinuric efficacy of AT1 receptor blockade in non-diabetic proteinuric patients in chapter 1.

Several recent studies have shown that in proteinuric patients the addition of aldosterone receptor blockade to ACEi or to an AT1 receptor blocker can lead to further reduction of proteinuria:\textsuperscript{34,35,58-61} this is discussed in more detail below.

The effects of renin-blockade as a novel mode of intervention in the RAAS are currently under investigation in the ALTITUDE study. This trial investigates the renoprotective potential of renin inhibition by aliskiren on top of RAAS blockade with either ACEi or AT1 receptor blockade on hard end points in diabetic renal patients.\textsuperscript{62}

Recent data paved the way for a broader adoption of statins in CKD. Two meta-analyses claimed improvement of renal outcome (effects on proteinuria and slowing of the decline in renal function loss) with statin treatment.\textsuperscript{26,63} A recent meta-analysis also showed that the addition of statins is associated with lipid lowering, cardiovascular, and antiproteinuric benefits in CKD in a setting of secondary prevention.\textsuperscript{21} Moreover, at different stages of CKD, statin treatment reduced cardiovascular risk and mortality in a similar fashion to that seen in trials of statins in non-CKD populations.\textsuperscript{21}

Finally, as outlined in more detail below, better monitoring tools of therapeutic efficacy to titrate renoprotective treatment are needed.
Pathophysiological effects of aldosterone and its escape from RAAS-blockade

Classically, aldosterone exerts its effects on volume status by the regulation of sodium absorption through the mineralocorticoid receptor (MR) on epithelial sodium channels, which are located on cortical collecting duct cells in the distal nephron. Angiotensin II, potassium and adrenocorticotropic hormone (ACTH) stimulate the adrenal zona glomerulosa to synthesize aldosterone. Circulating aldosterone then binds to the inactive cytosolic MR of target cells, resulting in translocation of the ligand-activated MR into the nucleus, where it binds to hormone-response elements in the regulatory region of target gene promoters (the MR is a nuclear hormone receptor). In the distal nephron of the kidney, MR induction of serum- and glucocorticoid-inducible kinase-1 (sgk-1) gene expression triggers a cascade that leads to the absorption of sodium and water through the epithelial sodium channel and potassium excretion with subsequent volume expansion and hypertension.64

Additionally, there is increasing evidence that aldosterone is directly involved in the development and progression of renal disease via nonepithelial mineralocorticoid receptor (MR) mediated effects.33;65-67 In vitro studies have confirmed that the pathological effects of aldosterone derive at least in part from its non-hemodynamic actions. Aldosterone exerts profibrotic effects through increased production of TGF-β, reactive oxygen species, PAI-1 and increased collagen gene expression and synthesis, which can be abolished by MR blockade.36;65;68-70 Animal studies have shed more light on the pathophysiological non-hemodynamic effects of aldosterone and the MR. In several models of nephropathy including spontaneously hypertensive stroke-prone rats MR antagonists markedly ameliorated glomerular and/or tubulo-interstitial injury without effects on systemic blood pressure or volume status.67;71 Exogenous aldosterone infusion completely reversed the renoprotective effects of ACEi in this model.67

ACEi and AT1 receptor blockers initially decrease aldosterone plasma concentration, but suppression cannot be sustained in all patients. A recent systematic review showed that the initial suppression of aldosterone was not sustained in 10% to 50% of patients treated with conventional RAAS blockade (ACEi and/or AT1 receptor blockers).72 This is particularly the case during long-term treatment73 or during sodium restriction,74 which potentiates the adrenal response to angiotensin II.75 Thus, many patients are exposed to aldosterone-escape-from-RAAS blockade, high levels of a hormone with known profi-
brotic actions on the kidney. Several recent studies indeed show renoprotective effects of aldosterone receptor blockade, either alone or when added to ACEi or AT1 blockade. In proteinuric patients the addition of aldosterone receptor blockade leads to further reduction of proteinuria.34,35,58-61 In non-diabetic proteinuric patients with CKD the reduction of proteinuria seen by the addition of spironolactone to ACEi, AT1 blockade or their combination, is related to aldosterone levels.34 This strongly suggests that aldosterone is a component of the renal damage that is associated with CKD and that its inhibition by ACEi, AT1 blockade or their combination can be incomplete.34

We investigated whether aldosterone has direct effects on the structural integrity of the glomerular basement membrane (GBM) in chapter 3. For the structural integrity of the GBM heparan sulfate proteoglycans are important.76 Proteoglycans are glycoconjugates consisting of a core protein to which linear glycosaminoglycan side chains are covalently attached.76 Loss of heparan sulfate in the GBM is commonly present in experimental and human glomerular diseases, especially during proteinuric conditions. This decreased expression of heparan sulfate can be attributed to an increased expression of heparanase, an endo-β(1-4)-D-glucuronidase that cleaves heparan sulfate side chains from proteoglycans. In several experimental and human glomerular diseases, such as passive Heymann nephritis (a model for membranous nephropathy), puromycin- and adriamycin-induced nephrosis, anti-GBM nephritis, diabetic nephropathy and minimal change disease, an increased expression of glomerular heparanase was observed, which correlated with a decreased expression of heparan sulfate in the GBM.41,77-84 Importantly, treatment with heparinoids, heparanase inhibitors and even inhibition of heparanase with specific heparanase neutralizing antibodies have been shown to reduce proteinuria.80-82,85 Moreover, in patients with type 1 and type 2 diabetes increased urinary heparanase activity is present, which is associated with the degree of albuminuria.86 In non-diabetic proteinuria, increased urinary heparanase activity is also present in patients with membranous glomerulonephritis.86 In these patients heparanase activity correlated with urinary β2-microglobulin excretion, a marker for tubular damage.86 Taking these results together, a role for heparanase in the pathogenesis of proteinuria induced renal damage is likely.

In nephrotic rats AT1 receptor blockade reduced glomerular heparanase expression and restored loss of heparan sulfate in the GBM.41 Moreover, diabetic patients treated with RAAS blockade had lower urinary heparanase activity of borderline significance compared to patients treated with other anti-hypertensive drugs, suggesting that components
of the RAAS are involved in the regulation of heparanase.\cite{86} Therefore, in chapter 3 we investigated whether the RAAS components aldosterone and angiotensin II can directly regulate heparanase expression \textit{in vitro}. Moreover, we studied whether optimization of antiproteinuric treatment with combined treatment of aldosterone receptor blockade and ACEi is associated with the most effective suppression of heparanase expression and consequent restoration of heparan sulfate in nephrotic rats in chapter 3.

In chapter 4 we studied whether aldosterone is involved in the pathogenesis of CAN. Ischemia/reperfusion injury at transplantation predisposes to the development of CAN.\cite{87,88} Recently, it was shown that MR blockade administered as spironolactone prior to the induction of renal ischemia/reperfusion injury prevented renal function loss, proteinuria and oxidative stress in rats.\cite{89} Moreover, MR blockade with spironolactone increased survival and prevented the progression of tubulo-interstitial fibrosis and arteriolar thickening in cyclosporine nephrotoxicity in rats.\cite{90} Since ischemia/reperfusion injury, interstitial renal damage and transplant arteriopathy play an important role in the development and progression of CAN, aldosterone blockade might protect against CAN. In chapter 4 we determined the efficacy of spironolactone in attenuating CAN after experimental renal transplantation in rats.

\textbf{Better monitoring tools of therapeutic efficacy: Kidney Injury Molecule-1}

To improve renoprotective treatment, it is of major importance to have optimal tools to monitor progressive renal function loss and the efficacy of intervention. Of course, the rate of renal function decline, as assessed by calculating the slope of serial serum creatinine based estimations of renal function, is a good predictor. However, for the monitoring of short term therapy response this is not convenient. Moreover, it is essential to intervene at an early stage of CKD, preferably before renal function starts to decline. For centuries, doctors have used the urine for noninvasive assessment of kidney damage. In CKD proteinuria is a main predictor of future renal function loss\cite{91,92} and accordingly, reduction of proteinuria has emerged as the main surrogate parameter for long-term renoprotection. However, the predictive value of proteinuria reduction for improvement of renal prognosis is far from perfect, since responders to antiproteinuric treatment may still show progressive renal function loss. Since in most renal diseases long-term renal outcome is determined by the severity of tubulo-interstitial involvement,\cite{16,17} it is logical to assume
that a marker for tubular damage could provide a better, or additional tool to monitor progressive renal function loss and the efficacy of intervention.

Kidney Injury Molecule-1 (KIM-1) is a sensitive marker for the presence of tubular damage. KIM-1 is not detectable in healthy kidney tissue, but tubular KIM-1 expression is significantly induced in acute renal damage as well as in CKD and CAN, where it is significantly associated with tubulo-interstitial damage and inflammation. In experimental and in human renal disease elevated urinary KIM-1 levels are strongly related to tubular KIM-1, indicating that urinary KIM-1 can be a valuable biomarker for the presence of tubulo-interstitial damage. Furthermore, urinary excretion of KIM-1 is an independent predictor of graft loss in renal transplant recipients, demonstrating its prognostic impact. Data from studies with other tubular damage markers suggest that the urinary excretion of these markers may have the potential to guide renoprotective intervention therapy. Since urinary KIM-1 is currently the most sensitive noninvasive indicator for tubular damage, this would make it an excellent candidate which may allow closer monitoring of therapy response. In chapter 2 we explored the effects of antiproteinuric treatment on urinary KIM-1 excretion in non-diabetic proteinuric patients.

Advanced glycation end products (AGEs)

Not only the RAAS and heparanase are involved in tissue remodelling, but also AGEs. In part two of this thesis we investigated renal accumulation of AGEs in CKD and CAN and whether intervention in AGE formation, targeting this specific pathway of tissue remodelling, can exert renoprotective effects in CKD and CAN.

Proteins in the body are continuously modified by non-enzymatic glycation reactions, also known as Maillard or browning reactions (Figure 2). AGEs were originally characterized in 1912 by Louis-Camille Maillard, a French food scientist. He noticed the ability of this yellow-brown fluorescent substances to form cross-links to and between amino groups of proteins. The formation of AGEs is initiated by a non-enzymatic reaction between a free amino acid group from a protein, mostly lysine, and a carbonyl group from a reducing sugar to form a freely reversible Schiff base. Once formed, the labile Schiff base rearranges to a more stable ketoamine, called Amadori product. The Amadori compounds are slowly degraded, in complex dehydration and condensation reactions via
dicarbonyl intermediates, to finally yield advanced glycation end products (AGEs) in an irreversible manner. The degree of this non-enzymatic glycation is mainly determined by glucose concentration and duration of exposure, which explains why AGEs accumulate on long-lived proteins.

Pentosidine is a well characterized, highly fluorescent AGE and cross-linking amino acid in which one molecule of arginine and one of lysine are bridged by a 5 carbon sugar, a pentose (hence its name) in an imidazo-pyridinium structure (Figure 3). Pentosidine accumulates in long-lived tissue with age. The rate of accumulation in skin collagen is inversely proportional to species life-span.

The strikingly elevated levels of AGEs in proteins of non-diabetic uremic patients indicates that factors other than hyperglycemia also determine the rate of AGE formation. Indeed, AGEs can also be formed via non-glucose pathways. These much faster occurring alternative routes are highly oxidative stress driven. Proteins may be modified either directly by reactive oxygen species with the eventual formation of oxidized amino acids or indirectly by reactive carbonyl compounds. Reactive carbonyl compounds are formed through autoxidation of carbohydrates. In this alternative, oxidative stress driven pathway of AGE formation, glyoxal, methylglyoxal and glycolaldehyde are precursors of glycoxidation products. Accelerated formation of AGEs is thus not only due to an increased concentration of substrate (glucose), but also to increases in oxidative stress. In end-stage renal disease, in CAN and in atherosclerosis, conditions with substantial oxidative stress, increased accumulation of AGEs has indeed been shown. It is of interest that atherosclerosis shares many similarities with focal and segmental glomerulosclerosis.
The kidney plays a key role in AGE clearance. Under physiological conditions pentosidine is freely filtered by glomeruli, reabsorbed in the proximal tubule where it is degraded or modified, and eventually excreted in the urine. Plasma concentration of AGEs is thus inversely proportional to the glomerular filtration rate and higher plasma AGE levels are related to elevated serum creatinine concentrations.

There are at least two mechanisms by which AGEs may contribute to tissue injury. First, AGE modification directly alters the structure and function of extracellular matrix proteins. Due to the cross-linking of long-lived proteins, AGEs cause an increase in tissue rigidity and prevent the affected tissue from remodelling. Second, AGEs modulate cellular functions through ligation of specific cell surface receptors. The receptor for AGEs (RAGE) is a 35 kD protein that belongs to a member of the immunoglobulin receptor superfamily. AGEs can also bind to oligosaccharyl transferase-48 (AGE-R1), 80K-H phosphoprotein (AGE-R2), galectin-3 (AGE-R3), scavenger receptors types I and II, CD36, and Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1). By binding to these receptors, AGEs can activate intracellular signal transduction systems with the consecutive generation of free oxygen radicals, leading to activation of the redox-sensitive transcription factor NF-κB and induction of NF-κB controlled genes which are involved in inflammation. RAGE itself is also upregulated by NF-κB. So, AGEs can favour their own production by creating a vicious circle in which AGEs induce a milieu of abundant oxidative stress increasing their own formation and upregulation of its receptor RAGE.

Pharmacological intervention in AGE formation has extensively been studied. Pyridoxamine (PM, Figure 4), a natural vitamer of the vitamin B6 family, is an effective inhibitor of AGE formation. PM decreases the oxidative stress driven formation of AGEs in three ways. First, it chelates metal ions that catalyze the oxidation reaction of Amadori compounds. Second, PM scavanges reactive carbonyl compounds with glycating properties such as glyoxal and glycolaldehyde. Third, PM can directly scavenge oxygen radicals by donating the hydrogen atom of the phenol group in PM itself. In these ways, PM blocks the oxidative degradation of the Amadori intermediate in
the Maillard reaction; hence its name post-Amadori AGE formation inhibitor. In experimental diabetes, pharmacological intervention in AGE formation with PM and aminoguanidine protects against renal structural lesions, proteinuria and renal function loss, supporting the therapeutic potential of AGE inhibition. Interestingly, PM not only provides renoprotection in diabetes, but also in normoglycemic obese Zucker rats, demonstrating that its beneficial effects are not limited to hyperglycaemia-related models of renal damage.

In chapter 5 of this thesis we investigated renal accumulation of the novel glycolaldehyde (GA) derived AGE GA-pyridine in biopsies from patients with diabetic and non-diabetic nephropathies. In chapter 6 we studied whether pentosidine accumulates in the kidney in adriamycin-induced proteinuria and second, whether this possible renal accumulation is attenuated by antiproteinuric treatment with ACEi. Moreover, to study whether abnormal intrarenal protein trafficking, might be involved in the alleged renal AGE accumulation, we investigated renal pentosidine in unilateral adriamycin-induced proteinuria and compared the proteinuric to the non-proteinuric kidney. We investigated renal AGE accumulation and the renoprotective potential of intervention in AGE formation in an experimental model for CAN in chapter 7. Chapter 6, showing increased renal accumulation of pentosidine in adriamycin induced proteinuria, provided a rationale for studying the renoprotective potential of PM in this model in chapter 8.

Interactions between the RAAS and AGES

Direct interactions between the RAAS and AGES have been shown in vitro and in vivo. The complex interactions between the RAAS and AGES are outlined in Figure 5. Simultaneous incubation of glucose and protein with ACEi or AT1 receptor blockers prevents the in vitro formation of AGES. ACE-inhibitors affect the production of AGE precursors by chelating transition metal ions, which catalyse the formation of AGES in the Maillard reaction, and inhibiting various oxidative steps in the process of glycoxidation, including the formation of free radicals. Infusion of AGE-modified-albumin alters key components of the intrarenal RAAS in vivo in healthy rats. The associated renal damage can be antagonized by AT1 receptor blockade. Moreover, angiotensin II infusion increases AGE accumulation in the kidney and induces renal hypertrophy which can be antagonized by PM. Furthermore, RAAS blockade by losartan ameliorates the rise
in serum AGEs in normotensive subtotaly nephrectomized rats. Because of these interactions between the RAAS and advanced glycation, we compared PM to standard antiproteinuric treatment with ACEi and investigated the effects of combined intervention in these two different pathways of tissue remodelling in chapter 8 in adriamycin induced proteinuria. Furthermore, AGEs increase epithelial sodium channel mRNA and protein expression, with enhanced sodium uptake in cortical collecting duct cells in a time and dose dependent way. Similarly, AGEs significantly stimulated serum-and glucocorticoid-inducible kinase-1 (sgk-1) mRNA expression and protein activity in a time and dose dependent manner. When these cells were co-transfected with a plasmid expressing mutant sgk-1, stimulation of the epithelial sodium channel activity by AGEs was significantly less. This suggests that AGEs activate epithelial sodium channels by stimulating sgk-1 in cortical collecting duct cells. Taken together, these results provide an interesting novel mechanism that could be involved in disturbances of sodium balance, i.e. AGE induced activation of epithelial sodium channels. However, we had concerns on the in vitro preparation of AGEs in this study. For instance, it has been shown that AGE preparations which were free of significant levels of endotoxin contamination, failed to induce proinflammatory cellular responses, whereas endotoxin did. Therefore, to substantiate this interesting hypothesis it would be important to exclude possible endotoxin mediated effects. Chapter 9 consists of the abstract of the original study by Chang et al. and the letter we wrote in response to this, stressing the importance of using endotoxin free AGEs.

Figure 5. Interactions between the RAAS and AGEs. RAAS, renin-angiotensin aldosterone system; AGE, advanced glycation end products; PM, pyridoxamine; ACE, angiotensin converting enzyme; AT1 receptor, angiotensin II type 1 receptor; AT2 receptor, angiotensin II type 2 receptor.
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