Advanced glycation end-products: Implications for diabetic and non-diabetic nephropathies

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Abstract

Glycation is the process whereby sugars bind to the free amine residues of proteins. These newly formed modified molecular species are known as ‘advanced glycation end-products’, or AGEs. AGE toxicity may occur through at least three mechanisms: interaction with the receptor for AGEs (RAGE); tissue deposition; and in situ glycation. AGEs trigger proinflammatory, profibrotic and procoagulant cellular responses that are capable of damaging tissues, often targeting particular organs. In diabetic patients, the conditions needed to promote AGE formation are all present, and are further accentuated by accompanying renal failure. The aim of this review is to outline the involvement of AGEs in the various forms of renal pathology associated with diabetic and non-diabetic nephropathies. AGEs are present in all renal compartments in diabetic patients, including the vessels, glomeruli, tubules and interstitium. Many cell types may be activated—specifically, endothelial, tubular and mesangial cells, and podocytes. AGEs play a major role in the accumulation of extracellular matrix, as occurs in diabetic glomerulosclerosis, and are also involved in most diabetic (renovascular, microangiopathic and glomerular) and non-diabetic renal injury associated with progressive glomerulosclerosis and ageing.

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Keywords: Advanced glycation end-products; Diabetic nephropathy; Glomerulosclerosis; Fibrosis; RAGE; Review

Résumé

Produits de la glycation avancée : implication au cours de la néphropathie diabétique et des autres néphropathies.


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Mots clés : Produits de glycation avancée ; Néphropathie diabétique ; Glomérulosclérose ; Fibrose ; RAGE ; Revue générale

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1. Introduction

Diabetes-induced renal disorders can affect multiple compartments of the kidney, as implied by the term ‘diabetic nephropathies’, including: the large vessels, with the subsequent development of atherosclerosis and renovascular disease; the microvessels, in the form of arterio- and arteriolar sclerosis; and, in particular, the glomeruli, leading to nodular or diffuse glomerulosclerosis, generally considered the quintessential lesion of diabetic nephropathy (DN) [1]. These pathological changes arise in part from the adverse effects of hyperglycaemia, leading to the formation of advanced glycation end-products (AGEs) [2,3]. AGEs are also implicated in the pathophysiology of non-diabetic renal diseases and ageing. They have been found in renal tissues in IgA and lupus nephritis, hypertensive nephropathy, renal fibrosis and chronic transplant rejection [4–6], and are also implicated in physiological ageing as well as age-related disorders such as Alzheimer’s disease and macular degeneration [7]. Diabetologists, along with nephrologists and geriatricians, need to be familiar with the deleterious effects of AGE formation and resulting organ damage. The present review is an update of recent advances in our understanding of the roles of AGEs in the development of DN, non-diabetic renal disease and renal ageing.

2. AGEs and their interaction with receptors

Chronic hyperglycaemia promotes the formation of modified molecular species such as AGEs. AGEs result from the binding of free lysine or arginine NH₂ residues on proteins to a sugar with an ‘ose’, such as glucose, fructose or glucose-6-phosphate. The formation of AGEs can occur through three major biochemical mechanisms: glycation; the polyol pathway and glycoxidation.

The first, glycation, directly depends on glucose concentrations, time and temperature, and requires several stages before an AGE can be formed. First described in 1912 by Louis-Camille Maillard at the Paris Academy of Sciences, glycation is a non-enzymatic process in which glucose binds to a protein in a high-glucose-concentration environment [8]. Glycation of proteins involves the formation of a Schiff base, which is then transformed into Amadori products that, in turn, are subject to further molecular modifications before becoming an AGE. The most familiar intermediary glycation product measured in routine clinical practice is glycated haemoglobin (HbA1c).

The second mechanism, the polyol pathway, is enzymatic in nature. Glucose is transformed by aldolase reductase or sorbitol dehydrogenase into an intermediary product that, after binding to a protein, becomes an AGE.

The glycoxidation pathway is based on oxidative stress, whereby the oxidation of glucose leads to the formation of gly-oxal and methylglyoxal. These oxidized sugars are extremely unstable and can quickly react with different proteins to form AGEs (Fig. 1).

Although there is no precise AGE classification, these molecules can be differentiated according to the type of binding, the nature of the glycated protein or the particular sugar that is bound. So far, two AGEs have been especially widely studied, as they are antigenic and easy to analyze: Nε-(carboxymethyl)lysine (CML), which is mainly present in biological fluids and has a short half-life; and pentosidine, which is mostly found in the extracellular matrix (ECM) and, thus, has a longer half-life. AGE toxicity is linked to three mechanisms: deposition; in situ glycation and receptor interaction.

2.1. AGE receptors

At least five types of AGE receptors have been identified so far: the macrophage scavenger receptor 1 (MSR1 or CD36) [9]; the AGE receptor R1 (AGE-R1 or p60), corresponding to oligosaccharyl transferase 48; the AGE receptor R2 (AGE-R2 or p90), which corresponds to phosphoprotein 80K-H; the AGE receptor R3 (AGE-R3 or galactin-3), a scavenger receptor that recognizes galactoside residues; and RAGE, commonly referred to as the ‘receptor for AGEs’ and the most widely studied type [10].

RAGE, a transmembrane receptor of the immunoglobulin superfamily [11], is a multiligand receptor with the capability of interacting with several types of proteins, including AGEs, the S100 proteins or calgranulins (belonging to the proinflammatory cytokines), the high-mobility group B1 (HMGB1) also known as ‘amphoterin’, and the proteins with a β fibrillar structure [12,13]. In humans, the RAGE gene is located on chromosome 6 in the major histocompatibility complex (MHC) class III region [14]. RAGE consists of an intracellular domain, a short transmembrane domain and an extracellular domain. Genetic variants of the RAGE gene (AGER in HUGO nomenclature) have been associated with vascular disease and risk of renal disorders [15]. The association between the RAGE -374 T/A homozygous AA genotype and cardiovascular disease as well as albumin excretion in type 1 diabetic patients with poor metabolic control suggests a gene–environment interaction in the development of DN and cardiovascular complications [16]. The 82 Ser allele of the RAGE gene is a risk allele for developing advanced nephropathy in Caucasian type 1 diabetic patients [17].
Soluble RAGE (sRAGE) corresponds to the extracellular domain of RAGE. As the N-terminal ‘V’-type domain is also included, sRAGE has the same ligand-binding specificity as RAGE and may act as a decoy by binding with proinflammatory ligands and preventing them from reaching membrane-associated RAGE. sRAGE comprises two forms: endogenous secretory RAGE (esRAGE) and cleaved RAGE (cRAGE; Fig. 2A) [18,19]. The former occurs via alternative splicing of intron 9/exon 10 of the RAGE gene [18]. Another mechanism responsible for the circulating pool of sRAGE is based on proteolytic processing (by ADAM10) from the full-length membrane-bound RAGE that produces cRAGE [19]. The formation of cRAGE can be inhibited with matrix metallopro-

2.2. Interaction of AGE and RAGE

The AGE/RAGE interaction activates a series of intracellular signalling pathways, including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nuclear factor kappa-B (NFkB), with production of reactive oxygen species (ROS) [25]. The cellular response can involve several types—proinflammatory, profibrotic, procoagulant and/or angiogenic—with overexpression of cell adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), and the production of cytokines [interleukin-2 (IL-2), IL-6 and tumour necrosis factor-α (TNF-α)], tissue factor (TF) and vascular endothelial growth factor (VEGF; Fig. 2B) [22,26–28].

3. AGEs in diabetic nephropathies

3.1. Where are AGEs located in the diabetic kidney?

AGEs can be identified in many renal structures. In arteries, AGE deposits are mainly found in atheromatous plaques. Their presence has been reported in the carotid, aortic, femoral, radial and iliac arteries. However, there are no reports in the literature of the presence of AGEs in the renal artery, although such a finding appears to be reasonable [29,30]. The AGE concentration in plaques is correlated with the degree of atherosclerosis, and is even higher in diabetic patients with renal failure [25,31]. An ultrastructural study carried out in rats with DN demonstrated the presence of AGEs in the glomerular basement membrane, mesangium, podocytes, tubules and endothelial cells [23,32]. The extent of AGE concentration is proportional to the severity of mesangial expansion in the course of DN [33] (Table 1).

3.2. Cellular effects of AGEs

Endothelial cells express RAGE, where interaction with AGEs can mediate numerous negative effects leading to macro- and microvascular endothelial dysfunction. Both in vitro and in vivo, endothelial RAGE activation leads to increased expression of VCAM-1 (blood cell–endothelium interactions) and E-cadherin (homotype adhesion) [34–36]. The glycation of endothelial intracellular proteins has also been described. In vitro, glycation of fibroblast growth factor-β (FGF-β) is accompanied by a significant decrease in binding of heparin, its natural ligand, and by a reduction in its mitogenic activity [37]. AGEs directly lower the level of nitric oxide synthase (NOS) expression [38]. They can also directly interact with NO and inactivate
in phenotype changes, with a decrease in epithelial markers and altered cell function favouring profibrotic activity. EMT results in myofibroblastic cells, a transformation that is accompanied by the transcription of numerous genes \[55\]. TGF-β signal transduction in tubular cells is followed by an increase of albuminuria, and diminution of ACTN-4 expression has been correlated with the degree of albuminuria and renal development of proteinuria, and diminution of ACTN-4 expression by podocytes is significantly diminished \[53\]. This alteration is an initial step in the development of renal macro- and microvascular diabetic lesions \[41,42\].

Mesangial cell RAGE activation has effects on the cell cycle and maintains mesangial cells in a quiescent state. In turn, this inhibition of cell proliferation promotes mesangial cell apoptosis and hypertrophy \[43,44\]. AGEs increase mesangial synthesis of fibronectin as well as collagen types I and IV. Following RAGE activation, mesangial cells secrete monocyte chemoattractant protein-1 (MCP-1), which participates in the inflammatory process \[44,45\]. Mesangial cells also express other receptors for AGE, such as AGE-R1 and AGE-R2, the activation of which may contribute to the damaging effects of AGEs by promoting mesangial cell apoptosis \[46,47\].

Podocyte RAGE activation is strongly implicated in the development of DN \[48,49\]. AGEs interfere with the podocyte cell cycle and inhibit cell proliferation, causing cytoplasmic hypertrophy followed by apoptosis. This effect can be prevented by RAGE small interfering RNA (siRNA) \[50\]. Glycated albumin, through the engagement of RAGE, inhibits nephrin synthesis by podocytes, and alters the slit diaphragm that forms the glomerular filtration barrier \[51\]. AGEs also interact with podocyte cytoskeletal protein α-actinin-4 (ACTN-4), a key component of the slit diaphragm \[52\]. In the presence of AGEs, ACTN-4 gene transcription and expression by podocytes is significantly diminished \[53\]. This alteration is an initial step in the development of proteinuria, and diminution of ACTN-4 expression has been correlated with the degree of albuminuria and renal failure \[54\].

AGE engagement with RAGE on tubular cells leads to TGF-β pathway activation. In vitro, RAGE activation by cultured epithelial tubular cells is followed by an increase of TGF-β concentration in the culture medium, and by enhancement of transcription and expression of small mothers against decapentaplegic-2 and -3 (SMAD-2 and SMAD-3). These two molecules, which are activated early in the process, affect the transcription of numerous genes \[55\]. TGF-β induces the epithelial-to-mesenchymal transition (EMT) process, which involves phenotype modification of epithelial tubular cells into myofibroblastic cells, a transformation that is accompanied by altered cell function favouring profibrotic activity. EMT results in phenotype changes, with a decrease in epithelial markers and the presence of mesenchymal markers such as α-smooth muscle actin, and plays a key role in the development of renal fibrosis by stimulating the secretion of ECM proteins such as collagen and fibronectin \[3\]. In vitro, tubular epithelial cell RAGE activation by AGEs induces EMT via the TGF-β pathway, whereas EMT may be totally inhibited by anti-RAGE antibodies and partially inhibited by anti-TGF-β antibodies \[56\]. In diabetic rats, 0.5–5% of tubular cells express myofibroblast markers. Renal biopsies from patients with DN also demonstrate the presence of EMT \[56\].

### 3.3. Effects of AGEs on ECM

Glycated proteins can modify ECM structure by cross-linking proteins such as collagen and elastin. This process increases ECM rigidity, reduces its turnover and might, at least partly, explain the vascular rigidity and hypertension that typically develop during diabetes and ageing \[57\].

DN progression is accompanied by significant mesangial expansion that is partly linked to a decrease in ECM degradation and reversible in the presence of aminoguanidine, an inhibitor of AGE formation \[58\]. Modification in matrix turnover includes regulation of MMP, and of their activators [membrane-type MMP (MT-MMP)] and inhibitors [tissue inhibitor MMP (TIMP)]. The collagen affinity for MMP-3 is clearly diminished when the latter is glycated \[59\]. While MMP-2 gene expression augments this activity, the presence of AGEs in the mesangium reduces the activity of MT1-MMP (an MMP-2 activator) by 45%. Also, there is a parallel augmentation of the activity of inhibitors TIMP-1 and TIMP-2, although these effects can be prevented by aminoguanidine, further emphasizing the important role of AGEs in disease progression \[60\]. MMP activity in non-collagen proteins such as MMP-7 is also lowered in the presence of AGEs \[61\] (Fig. 3). ECM glycation leads to alterations in the cell–matrix interaction. In vitro, the culture of podocytes on a glycated matrix is accompanied by a reduction in cell attachment and proliferation \[62\]. AGEs also regulate ECM protein and protease expression by human mesangial cells \[8\].

### 3.4. From animal models to humans

The presence of AGEs in the kidney is the result of different mechanisms: trapping; \textit{in situ} glycation and tubular reabsorption. Compared with control animals, intravenous injection of AGEs over a 5-month period in normal rats led to a 50% increase in renal accumulation of AGEs \[63\]. AGE distribution was comparable to that observed in DN and mimicked the histological changes of DN, including hypertrophy and sclerosis of glomeruli, mesangial matrix hypertrophy, thickening of

### Table 1
AGE deposits in diabetic kidneys, as reflected by the percentage of AGE-positive staining in type 2 diabetic renal biopsies.

<table>
<thead>
<tr>
<th>AGE</th>
<th>Mesangium</th>
<th>Glomerular basement membrane</th>
<th>Tubular basement membrane</th>
<th>Interstitium</th>
<th>Vessel walls</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA-CML (%)</td>
<td>96</td>
<td>4</td>
<td>85</td>
<td>19</td>
<td>96</td>
</tr>
<tr>
<td>Pentosidine (%)</td>
<td>77</td>
<td>4</td>
<td>31</td>
<td>90</td>
<td>54</td>
</tr>
</tbody>
</table>

Data are based on a study of 26 human PBR \[27\]. HSA-CML: human serum albumin-carboxymethyl-lysine.
4. AGEs and diabetic nephropathy: similarities with other nephropathies and ageing

DN is not the only kidney condition involving AGEs. Renal disorders linked with AGEs are also reported during CRF, chronic inflammatory diseases, high dietary AGE and ageing, although the precise role of AGEs in these different kidney pathologies has yet to be elucidated. During diabetes, AGEs are a consequence of chronic hyperglycaemia and precede diabetic complications [70]. In non-diabetic patients, their presence could disrupt the equilibrium between dietary intake, oxidation (glycoxidation), in situ glycation and renal elimination. Excessive kidney AGE deposition is found in arterionephrosclerosis, IgA nephropathy, lupus nephritis, uraemia and the experimental murine model of focal sclerosis mediated by adriamycin [23,71]. Compared with DN, AGEs and their distribution often differ in these diverse pathological settings (Table 2).

All biopsies performed in patients with arterionephrosclerosis have been strongly positive for CML levels in the glomeruli and vascular structures, with pentosidine being mostly present in the interstitium [23]. AGEs are known to be important in atherosclerosis progression and are positively correlated with cardiovascular mortality in non-diabetic women [72]. During IgA nephropathy, CML and pentosidine staining in the mesangium and interstitium is particularly mild, whereas these two types of AGEs are found in excess in the tubules. Also, there are high levels of pyrraline in the interstitium, while vascular staining for AGEs is faint [33,73]. In lupus nephritis, pathological lesions are present in the glomeruli and, in some cases, the vessels, too, with a similar distribution, but weaker intensity of staining for AGEs compared with DN. The increased AGEs found in lupus glomerulonephritis could be the consequence of chronic inflammation that leads to oxidative stress and glycoxidation [74]. In vitro, RAGE activation of mesangial cells by DNA antibody induces a proinflammatory response and may contribute to renal changes [75].

It is uncertain to what extent kidney AGE accumulation is a cause or consequence of kidney disease, and AGEs could even exert effects through a sort of vicious circle. During CRF, uraemia itself promotes blood and tissue AGE accumulation. Reduction of AGE clearance and permanent oxidative (carbonyl) stress are responsible for such AGE excess [65]. Also, in CRF, the presence of AGEs increases endothelial dysfunction associated with an imbalance between relaxation and vasoconstriction: endothelial reactivity is decreased, and the response to ischaemia and hyperthermia is not adaptive [76]. In 51 non-diabetic uraemic patients, blood levels of AGEs and mRNA of tubular and glomerular basement membranes, and effacement of podocyte foot processes. AGE renal accumulation was accompanied by increased excretion of both total urinary protein (2.5 times that of the controls) and urinary AGE. Injection of AGEs into non-diabetic rats that had undergone subtotal nephrectomy led to glomerulosclerosis and tubulointerstitial damage [63,64]. A study of rats given radioactive pentosidine confirmed the key role of the kidney in AGE metabolism, with 83% of the total radioactivity eliminated via the urine, and a large residual quantity of AGEs trapped in the kidneys—35% compared with only 2% in the liver [65]. Urinary elimination of AGE is directly dependent on dietary intake. Indeed, a reduced AGE intake was followed by lower circulating and tissue AGE levels in mice. In healthy volunteers, a low-AGE diet was followed by a decrease in pyrraline urinary concentration [66]. These findings have also been reported in patients with chronic renal failure (CRF) and diabetes [67,68]. In addition, tubular reabsorption plays a key role in renal AGE accumulation. Epithelial cells in the proximal tubules express an apical membrane receptor called ‘megalin’, which can reabsorb filtered low-molecular-weight (LMW) proteins, including LMW AGEs. After megalin-binding, AGEs undergo endocytosis, and are then directed towards lysosomes through a mechanism that is both limited and saturable [65,69].

Table 2

<table>
<thead>
<tr>
<th>Mesangium</th>
<th>Glomerular basement membrane</th>
<th>Tubular basement membrane</th>
<th>Interstitium</th>
<th>Vessel walls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy (n = 26)</td>
<td>CML</td>
<td>CML</td>
<td>CML</td>
<td>Pentosidine</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis (n = 18)</td>
<td>Pentosidine</td>
<td>CML</td>
<td>Pentosidine</td>
<td>CML</td>
</tr>
<tr>
<td>Hypertensive nephropathy (n = 7)</td>
<td>CML</td>
<td>CML</td>
<td>CML/Pentosidine</td>
<td>CML</td>
</tr>
<tr>
<td>IgA nephropathy (n = 15)</td>
<td>MDA-lysine</td>
<td>ND</td>
<td>Pyrraline</td>
<td>MDA-lysine</td>
</tr>
<tr>
<td>Lupus nephritis (n = 11)</td>
<td>CML</td>
<td>CML</td>
<td>Pentosidine</td>
<td>CML</td>
</tr>
</tbody>
</table>

CML: carboxymethyl-lysine; MDA-lysine: malondialdehyde-lysine; ND: no determination; main localizations are shown in bold [27,33,63].
RAGE correlated with the glomerular filtration rate and endothelial dysfunction. AGEs accelerate degradation of renal function by promoting fibrosis at least in part through EMT, which itself has been correlated with the extent and severity of interstitial fibrosis and renal failure [77]. The postulated mechanisms of senescence and ageing in organisms, ranging from yeast to mammals, include environmental and genetic factors, elevated oxidative stress, cumulative DNA damage, altered gene expression, telomere shortening and energy utilization. As AGEs are implicated in physiological ageing, diabetes might be considered the equivalent of accelerated ageing, particularly at the level of the vascular tree [7]. AGE blood levels and tissue accumulation are increased in organs (blood vessels, brain, eyes and kidneys) in healthy elderly people [21,78–80], and AGEs also accumulate in various tissues with ageing. In Alzheimer’s disease, AGEs have been found in neurophilbrillary tangles and β-amyloid plaques [81,82]. AGEs also have direct cellular toxicity and are able to induce apoptosis through the caspase pathway [83,84]. As glycation of tau is followed by reduction of its solubility and ability to be degraded, this may represent one of the first steps in the development of Alzheimer’s [85]. Also, as many cells and tissues of the eye are profoundly influenced by glycation, AGEs are now receiving considerable attention as a possible pathogenic factor in vision disorders. AGEs accumulate in the macula in age-related macular degeneration, and RAGE activation in retinal pigment epithelial cells may contribute to the up-regulation of VEGF, thereby potentially inciting or propagating neovascular macular disease [86]. In addition, numerous factors, including AGEs, can contribute to renal ageing. Through AGE accumulation, in situ glycation and RAGE activation, glycation could promote the physiological and pathological effects of renal ageing. It has recently been shown that AGEs in the diet correlate with serum AGE levels, oxidative stress, lifespan and renal dysfunction. Indeed, exogenous (dietary) AGEs are reported to accelerate ageing in mice, while the addition of a chemically defined AGE to low-AGE mouse chow can predispose to the development of cardiovascular and chronic kidney diseases [87].

5. Prevention and treatment

Diet may play a key role in the prevention of AGE-linked pathologies. Food acts as a dietary source of variable amounts of exogenous AGE, depending on its sugar and protein composition, and how it is cooked. The richer in proteins and sugars a food is, and the more it is heated, the more AGEs it will contain. In murine models, food restriction or a diet low in AGE protects against the development of renal lesions [87,88]. Maintaining optimal glycaemic balance provides a way of limiting endogenous AGE neoformation.

Several targeted molecules have been tested as AGE inhibitors. Aminoguanidine interacts with glucose-derived products obtained from glycoxidation and the polyol pathway. Its beneficial effect has, to a great extent, been demonstrated in vitro and in vivo in animal models, with significant reduction of AGE levels in blood and tissues [89,90]. In diabetic mice, aminoguanidine not only prevents DN, but also diabetic retinopathy and neuropathy, and improves endothelial dysfunction [91,92]. ACTION 1 (Aminoguanidine Clinical Trial in Overt Type 1 Diabetic Nephropathy), a phase-III clinical trial, failed to demonstrate any significant differences between placebo and aminoguanidine-treated groups, and only a trend towards lower serum creatinine and lower urinary protein was observed with the treatment. The study was stopped because of severe adverse effects such as abnormal liver function tests, gastrointestinal disturbances, flu-like symptoms and vasculitis or lupus-like phenomena [91].

Another AGE inhibitor has also been studied—namely, 2-isopropyldiethylenebrozono-4-oxo-thiazolidin-5-ylacetamide (OPB-9195), an ROS chelator that prevents the formation of glucose derivatives such as methylglyoxal, glyoxal and 3-deoxyglucosone, intermediaries that are indispensable for AGE formation [93,94]. OPB-9195 is able to slow the progression of glomerulosclerosis through a reduction in glycated collagen. However, clinical studies were stopped because of vitamin B6 deficiency induced by OPB-9195 (pyridoxal chelator).

Of the aromatic ‘LR’ group, LR-90, LR-9 and LR-74 all inhibit glycation of Amadori products and block glycoxidation. Their in vivo effects are comparable to those of aminoguanidine and OPB-9195 [95]. Pyridoxamine, a vitamin B6 derivative, inhibits the transformation of intermediary glycation products [96]. Following injection of pyridoxamine in diabetic rats, a decrease in the progression of DN has been observed [97]. Pyridoxamine has also been used in phase-II clinical trials without major side-effects [98,99]. Furthermore, benfotiamine, a liposoluble vitamin B1 derivative, acts on glucose-derived products and reduces the glycation of albumin [100]. Benfotiamine can reduce AGE formation in endothelial cells and prevent the development of DN lesions [101]. In type 2 diabetic patients, oral benfotiamine prevents endothelial dysfunction induced by a rich AGE diet [102].

There are several possible ways to limit tissue AGE accumulation and reduce their deleterious effects. Alagebrum (ALT-711) is an AGE cross-link breaker (sugar/protein cross-link) that can reduce AGE accumulation and improve endothelial dysfunction [103]. ALT-711 administration in diabetic mice prevented the biological and histological changes of DN [104]. Several clinical trials have also demonstrated beneficial effects of ALT-711 on blood pressure, arterial stiffness, left ventricular mass and diastolic filling [105–107].

In addition, a number of treatments currently in use as targeted therapies for various disorders have recently been reported to have ‘anti-AGE’ properties, including renin–angiotensin–aldosterone system blockers [angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs)], statins, some oral antidiabetics and antioxidative molecules. In vitro, ACE inhibitors and ARBs are able to block the secretion of AGE-induced TGF-β and reduce the formation of ROS [108]. These agents can also increase blood concentrations of sRAGE, which can bind AGEs, thereby reducing renal AGE deposits and DN progression [109,110], and have beneficial effects on oxidative stress, reducing the glycoxidation pathway [111]. Statins can reduce RAGE expression in diabetic patients, leading to effects on MCP-1 production [112]. In vitro, metformin and piogli-
tazone were able to reduce AGE formation, but these effects are yet to be confirmed in vivo [113–115]. Furthermore, some antioxidants have demonstrated antiglycation effects [116].

Other compounds that are currently only used for basic research may be developed for therapeutic purposes. The most promising candidates are sRAGE and anti-RAGE antibody. sRAGE acts as a decoy by binding AGEs and blocking RAGE activation and, when injected intraperitoneally into db/db mice, prevents albuminuria, glomerulosclerosis and glomerular base-

Anti-RAGE antibody specifically blocks RAGE activation. In vitro, RAGE antibodies reduce ECM remodelling [6]. Administration of anti-RAGE antibody over a 2-week period reduced serum creatinine, albuminuria, GBM thickness and mesangial volume in db/db and type 1 diabetic mice [119,120].

6. Conclusion

AGEs have numerous implications in ‘diabetic nephropathies’. However, although they play a major role in the development of DN itself, their presence has also been demonstrated in other renal diseases and in ageing. The battle against AGEs constitutes an integral part of the current recommendations for renoprotective and ‘future’ anti-ageing strategies. Nevertheless, while awaiting the development of a patient-specific, targeted therapeutic approach, there is still a need for strict glycemic control, a suitably adapted diet, and vascular protection through the use of ACE inhibitors or ARBs, statins and certain oral antidiabetic agents.

7. Conflicts of interest

No potential conflicts of interest relevant to this article have been reported.

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References


[3] Simonson MS. Phenotypic transitions and fibrosis in diabetic nephropa-

[4] Bohlender JM, Franke S, Stein G, Wolf G. Advanced glycation end-


