

Advanced glycation end products and the kidney

Jürgen M. Bohlender, Sybille Franke, Günter Stein, and Gunter Wolf

Department of Internal Medicine III, Friedrich-Schiller-University, Jena, Germany

Bohlender, Jürgen M., Sybille Franke, Günter Stein, and Gunter Wolf.

Advanced glycation end products and the kidney. *Am J Physiol Renal Physiol* 289: F645–F659, 2005; doi:10.1152/ajprenal.00398.2004.—Advanced glycation end products (AGEs) are a heterogeneous group of protein and lipids to which sugar residues are covalently bound. AGE formation is increased in situations with hyperglycemia (e.g., diabetes mellitus) and is also stimulated by oxidative stress, for example in uremia. It appears that activation of the renin-angiotensin system may contribute to AGE formation through various mechanisms. Although AGEs could nonspecifically bind to basement membranes and modify their properties, they also induce specific cellular responses including the release of profibrogenic and proinflammatory cytokines by interacting with the receptor for AGE (RAGE). However, additional receptors could bind AGEs, adding to the complexity of this system. The kidney is both: culprit and target of AGEs. A decrease in renal function increases circulating AGE concentrations by reduced clearance as well as increased formation. On the other hand, AGEs are involved in the structural changes of progressive nephropathies such as glomerulosclerosis, interstitial fibrosis, and tubular atrophy. These effects are most prominent in diabetic nephropathy, but they also contribute to renal pathophysiology in other nondiabetic renal diseases. Interference with AGE formation has therapeutic potential for preventing the progression of chronic renal diseases, as shown from data of animal experiments and, more recently, the first clinical trials.

RAGE; diabetic nephropathy; progression of renal disease; inflammation; glycoxidation

REDUCING SUGARS MAY REACT nonenzymatically with amino groups in proteins or lipids, involving a series of complex biochemical events with oxidative and nonoxidative molecular rearrangements termed the Maillard reaction that ultimately lead to stable covalent adducts known as advanced glycation end products (AGEs). The reaction proceeds slowly through different stages, leading to alterations of protein structure and molecular surface topology that profoundly change the affected molecule's biochemical properties. Using antibodies raised against AGE-specific epitopes, AGEs have been detected in human tissues under physiological conditions where their concentrations increase with chronological age, thereby linking AGEs to long-term protein degeneration and biological aging (36). Virtually any protein can become a target of glycoxidative modifications *in vivo*. Long-lived proteins, however, including matrix and structural proteins in tissue compartments with slow biological turnover, such as the lens or extracellular matrix (ECM), are particularly prone to AGE modification under conditions that favor their generation. AGE accumulation in tissues may result from accelerated AGE generation, decreased AGE degradation, or increased survival of AGEs. Increased tissue or plasma AGE concentrations have been detected in a variety of common diseases, including diabetes mellitus, chronic renal failure, atherosclerosis, arterial hypertension, and Alzheimer's disease, whereas their pathophysiological implications are only recently becoming understood in

more detail. Furthermore, AGEs are found also in primary rheumatoid arthritis and during osteoporosis. These findings underscore the universal pathogenetic role of AGEs (48, 138). Following the recent detection of specific receptors that recognize AGE-modified proteins (RAGE), activate intracellular signaling cascades, and finally orchestrate a plethora of proinflammatory and profibrotic cellular responses, current research has centered on the pathophysiological implications of AGE-RAGE interactions in a rapidly growing field of biomedical research (119).

AGEs and their receptors have now been established as a major pathogenic factor promoting complications of diabetes mellitus. In addition, they are also intrinsically involved in the pathophysiology of many other nondiabetic renal diseases. The pivotal role of AGEs during glomerular and tubulointerstitial damage and the development of uremic complications is now increasingly recognized as is their role in amplifying local immune and inflammatory responses in the kidney. In this context, the kidney is not only a target of AGEs but also a culprit because declining renal function entails a rapid increase in plasma concentrations of these products, thereby setting the stage for a vicious circle with deleterious consequences for the organ's survival. A complete review of the burgeoning literature dealing with AGEs and AGE-specific diseases is beyond the scope of this contribution. The interested reader is referred to several excellent reviews on these topics (7, 85, 120, 128, 151, 152). Here, we will focus on research related to increased AGE generation in uremic conditions and to the AGE-RAGE interaction in the kidney under physiological and pathological conditions.

Address for reprint requests and other correspondence: G. Wolf, Klinik für Innere Medizin III, Universitätsklinik Jena, Erlanger Allee 101, Jena, Germany (e-mail: Gunter.Wolf@med.uni-jena.de).

FORMATION OF AGES AND "CARBONYL STRESS"

The basic biochemical steps leading to the generation of AGEs are shown in Fig. 1. The initial reaction between reducing sugars and a protein proceeds through the formation of a labile Schiff's base, a rapid and easily reversible reaction that may halt at this point. The reaction product, however, may eventually continue to isomerize while slowly forming a ketoamine adduct termed the Amadori product. A typical Amadori compound, for instance, is hemoglobin A1c being used as a classic long-term marker for glycemia in diabetes mellitus. Such early products can undergo further complex oxidative decompositions, condensation, and additional molecular rearrangements that ultimately lead to stable, irreversible late AGEs, a process that may take weeks or months to accomplish. To fuel this process, glucose is an ubiquitous and predominant substrate, but other sugar molecules such as fructose, threose, glucose 6-phosphate, and glyceraldehyde 3 phosphate may also react similarly with proteins and produce chemically distinct AGEs according to the reaction partners, respectively (87, 128). Some important AGE compounds are listed in Table 1.

In diabetes, hyperglycemia leads to the activation of alternative intracellular metabolic pathways (e.g., polyol pathway) that foster the generation of these sugar moieties. N^ε-carboxymethyllysine (CML), N^ε-carboxyethyllysine (CEL), and pentosidine are major representatives of this class of AGEs. Furthermore, pentosidine is a cross-linking molecule that covalently bridges distant lysine and arginine residues in proteins by a complex C₅-ring, thereby linking different proteins together or forming intramolecular covalent bonds. Alternatively, these adducts can arise from intermediate lipid metabolism, for instance, by oxidation of polyunsaturated fatty acids and arachidonic acid involving metal ion-catalyzed reactions. Protein adducts arising from this pathway are accordingly termed advanced lipoperoxidation end products (ALEs). Compounds such as malondialdehyde (MDA)-lysine, hydroxynonenal (HNE)-lysine, or acrolein adducts derived from oxidized

Table 1. Biologically important AGEs (3)

Non-cross-linking monolysyl adducts
N ^ε -carboxymethyllysine (CML)
N ^ε -carboxyethyllysine (CEL)
Pyrraline
Cross-linking bislysyl adducts
Glyoxal-derived lysine dimer (GOLD)
Methylglyoxal-derived lysine dimer (MOLD)
3-Deoxyglucosone-derived lysine dimer (DOLD)
Hydroimidazolones
Glyoxal-derived hydroimidazolone (G-H)
Methylglyoxal hydroimidazolone (MG-H)
3-Deoxyglucosone-derived lysine dimer (3DG-H)
Other cross-linking compounds
Pentosidine*

AGEs, advanced glycation end products. *Forms intermolecular links between arginine and lysine residues.

hydroxy-amino acids, L-serine or L-threonine, belong to this class of terminally modified proteins. The term AGE is frequently used for both groups of compounds, and for reasons of simplicity this practice will be maintained here. Glycooxidative and lipoxidative pathways may independently result in the same end product CML, whereas pentosidine, for instance, is only formed from carbohydrate precursors. In contrast, AGEs such as imidazolone and pyrraline can also be generated independently from oxidative stress. Nonoxidative chemistry, finally, is involved in the generation of AGEs from methylglyoxal, for instance, during nonoxidative anaerobic glycolysis or based on 3-deoxyglucosone released during Amadori rearrangements (84, 85, 87, 101). Interestingly, these reactions are strongly influenced by the individual genetic background, although the genes responsible for this feature still remain unknown. Leslie and collaborators (66) performed a classic study in 39 monozygotic and 45 dizygotic otherwise healthy nondiabetic female twins. They showed that correlations for serum CML levels were higher in monozygotic ($r = 0.71$)

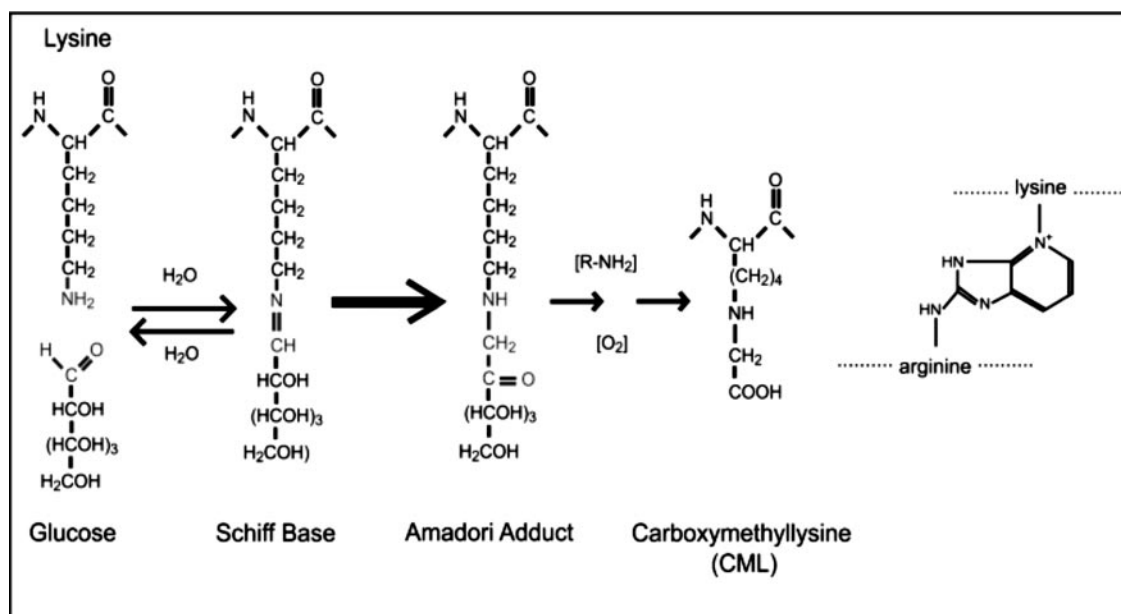


Fig. 1. Principal steps leading to advanced glycation end product (AGE) formation for N^ε-carboxymethyllysine (CML). The process may take weeks to months to accomplish. Pentosidine is a cross-linking AGE that links two molecules. Pentosidine is also found in its free form. RAGE, receptor for AGE.

compared with dizygotic twins ($r = 0.50$). The overall heritability was calculated at 74%, independent of genes influencing fasting glucose and hemoglobin A1c levels.

Diabetes mellitus and uremia are both conditions where significantly increased concentrations of AGEs are found in tissues and in plasma (72). Plasma pentosidine concentrations, for instance, may be up to 10-fold higher in patients with end-stage renal disease compared with normal subjects (81, 135). In diabetic patients, elevated glucose levels could readily explain their propensity to form AGEs, for instance, by shifting the biochemical reaction equilibrium toward glycation (Schiff's reaction) in a concentration-dependent manner, followed by irreversible AGE formation. Diabetics, however, are also characterized by chronic hyperlipidemia. Recently, Metz et al. (77) were able to demonstrate that diabetic animals treated with pyridoxamine to inhibit AGE formation produced considerable amounts of ALEs derived from polyunsaturated fatty acids. Treated animals furthermore excreted increased amounts of pyridoxamine-linked intermediates derived from fatty acids and arachidonate in their urine, indicating a pharmacological trapping mechanism. In their study, they provided experimental evidence that ALE formation is an important alternative pathway that quantitatively contributes to the accumulation of carbonyl-modified proteins in diabetes.

In uremic patients, AGE levels in plasma and tissues increase independently of glycemia because diabetic and nondiabetic patients on chronic maintenance hemodialysis show similar total concentrations of serum AGEs such as pentosidine, CML, and ALEs. Additionally, AGE levels in uremic patients on hemodialysis were found to be several times higher than in patients with diabetes and a normal kidney function. Mechanisms other than hyperglycemia therefore must be involved additionally (81, 84). In uremic plasma, accumulation of low-molecular-weight reactive carbonyl compounds such as 3-deoxyglucosone, dehydroascorbate, glyoxal, methylglyoxal, malondialdehyde, and arabinose derived from carbohydrate, lipid, or amino acid metabolism has been noted as a general feature. The presence in uremia of these reactive AGE precursors, termed reactive carbonyl compounds (RCO), together with carbonyl-modified proteins (AGEs), has therefore been summarized as a state of uremic carbonyl stress and pinpointed as a major pathogenic mechanism and risk factor for uremic end-organ damage (85, 87). Plasma pentosidine concentrations, for instance, have been shown to correlate directly with the amount of generated carbonyl precursors and may serve as a simple integral marker of uremic carbonyl stress. Uremic plasma, on long-term *in vitro* incubation, shows increased formation of pentosidine compared with nonuremic plasma, documenting its higher autoreactive potential. Addition of OPB-9195, an inhibitor of protein-RCO interaction, to uremic plasma samples inhibited pentosidine formation, supporting this pathogenic concept (82). One primary origin of increased carbonyl stress is oxidative stress, as observed by an increased amount of oxidized compounds and markers of oxidative stress in uremic plasma such as oxidized ascorbate (dehydroascorbate) or oxidized glutathione, which may subsequently contribute to increased RCO formation (85, 89). The relevance of this mechanism is underscored by the observation that AGEs are generated also in nonuremic normoglycemic animal models at sites of increased local oxidant stress or in situations with decreased antioxidant capacity, for instance, during the devel-

opment of arteriosclerotic lesions or plaque. Local generation of peroxynitrite from nitric oxide (NO) may contribute to this effect (95). Furthermore, accumulation of AGEs can be found at sites with endothelial injury with inflammatory neointimal proliferation but also during chronic arterial wall remodeling in arterial hypertension or during development of diabetic glomerular disease (91, 138, 171). Finally, myeloperoxidase-dependent generation of reactive oxygen species (ROS) by mononuclear cells at sites of inflammation may stimulate local AGE formation as an active cell-dependent process. For instance, macrophages releasing ROS into the intercellular space may foster extracellular AGE generation in their vicinity (6). Intracellular accumulation of AGEs increased directly with generation of ROS in endothelial cells (45). During chronic renal failure, there is a decreased ability of the body to clear reactive carbonyl compounds from the circulation, which contributes to globally increased RCO stress in this situation. The defective physiological RCO clearance in uremia is a consequence of a decreased availability of detoxifying scavenger molecules or RCO-degrading enzymes, together with a reduced clearance of RCO into the urine by the failing kidney itself. The lack of glutathione and its replenishing redox coenzyme NADPH as well as the markedly reduced concentrations of many other glutathione-dependent enzymes are a characteristic of uremia and are inversely related to high plasma pentosidine concentrations, the integral RCO marker (145). In patients with progressive renal failure, plasma pentosidine concentrations have been shown to increase in parallel with declining kidney function, supporting this pathophysiological concept (72, 135).

Using size-exclusion chromatography and fluorescence techniques, three main AGE fractions in uremic plasma have been found at a predominant molecular size of ~70, ~14, and <2 kDa, respectively (51). Pentosidine and CML are the prevalent AGE species, and most of their concentration is bound to serum albumin (~90%) displaying a molecular weight of ~69 kDa (81, 84). These high-molecular-weight AGEs, therefore, cannot be cleared by hemodialysis or glomerular filtration in significant amounts while the effect of different dialysis modalities on total plasma AGE concentrations is generally limited (132). Hemodialysis instead allows quantitative elimination of their much smaller reactive carbonyl precursors, with a typical molecular weight <5,000 Da (82, 102). The remaining serum AGE fraction represents free pentosidine and AGEs bound to small peptides of low molecular weight, which may appear in the urine depending on their seize. After being filtered into the urine, small AGE molecules may undergo further degradation and modification while being reabsorbed partly by proximal tubular cells (9, 80). Low-molecular-weight AGE peptides, however, may possibly have a higher toxic potential for tubular cells than the larger AGE proteins (34). Interestingly, after kidney transplantation the long-lived high-molecular-weight AGE fraction does not disappear rapidly from the circulation despite marked improvement of renal function. The quantitatively very small low-molecular-weight AGE fraction, however, decreases immediately, which has been attributed to improved renal AGE excretion and increased renal metabolism (41, 83). Three years after transplantation, specific AGE compounds, however, may decrease to serum levels found in chronic renal failure. Furthermore, in a recent study there was no apparent association of

serum AGE levels found in chronic renal failure with cardiovascular risk (131). Taken together, the reduced metabolic clearance, increased oxidative stress, and a higher rate of RCO formation are synergistically responsible for the high concentrations of AGEs found during chronic renal failure.

AGEs AND RECEPTOR-INDEPENDENT EFFECTS ON MATRIX PROTEINS

Carbonyl-modified proteins may have direct, receptor-independent pathological effects depending on the site of their generation. AGE-degenerated proteins may profoundly alter the structural, mechanical, and functional properties of the affected tissues. Cross-linking by AGEs has been noted for collagen molecules under a variety of pathological conditions, which, on multiplying their cross bridges, increase their biomechanical stiffness and brittleness. This feature, for instance, has profound implications for vascular wall elasticity and the development of arterial hypertension and its complications such as stroke (21, 158). AGE-modified collagen molecules have been implicated as an important pathogenic factor during subcutaneous skin aging. When the composition of glomerular extracellular matrix and capillary basement membrane is affected, organ dysfunction and failure may follow at an accelerated rate (36, 138). Such AGE-modified structural proteins can interfere with normal cell-matrix contact or inhibit physiological cellular growth and intercellular contact that are necessary to maintain tissue integrity and normal function. Endothelial cells may become insensitive to exogenous stimuli and develop abnormal growth patterns (12, 49). Albumin is one of the main targets of AGE modification in plasma. Its glycation, for instance, induces abnormal refolding of its three-dimensional structure, conformational rearrangement of amino acids into β -sheets and a cross- β structure characteristic of amyloid fibrils may result from this process as evidenced by transmission electron microscopy. Such glycated albumin subsequently is prone to condensate into amorphous fibrous aggregates while losing its innate biochemical properties (15). Another protein with a similar fate is β_2 -microglobulin (18). Local deposits of amyloid albumin condensates may be resistant to removal by macrophages and promote inflammation.

Pathological aggregates of AGE proteins may exhibit insensitivity to matrix metalloproteinase degradation, thereby causing abnormal extracellular matrix turnover or fibrous expansion of the interstitial space, with progressive scarification (93). AGE formation, nevertheless, is not restricted only to extracellular proteins accessible to circulating oxidative or carbonyl stress but has been also shown to occur intracellularly. Giardino et al. (44), for instance, showed that intracellular basic fibroblast growth factor produced by endothelial cells may rapidly undergo AGE modification following cytosolic oxidative stress, thereby changing its growth factor properties and reducing mitogenic cellular activity. Other cytokines and cellular proteins may similarly lose their intrinsic biological activity as a result of AGE modification. Intracellular AGE formation, finally, corresponds directly with hyperglycemia-induced intracellular oxidative stress and increased mitochondrial superoxide generation. As a result, methylglyoxal production is induced intracellularly, being the major AGE-forming reaction partner within endothelial cells. As a consequence, intracellular AGE concentrations rise progressively with critical damage of

vital proteins and cellular structures while causing cellular dysfunction (45, 99, 124). Another serious side effect is genomic DNA damage following intracellular carbonyl stress (133). This important intracellular AGE-generating pathway is operative independently of receptor-mediated effects and appears as a crucial pathophysiological step in AGE-associated renal and vascular damage in diabetes. Based on this concept, promising new pharmacological interventions to interrupt this intracellular sequence of events have been proposed recently (47). Similarly, angiotensin can induce intracellular oxidative stress via activation of its angiotensin type 1 receptor (AT₁R). In a transgenic rat model with chronic angiotensin-induced renal damage, we were recently able to demonstrate AGE formation and accumulation of CML in glomeruli, particularly within the outer glomerular linings under nondiabetic conditions (see Fig. 4) (2, 13). Our findings extend those by Nishikawa et al. (99) and others (163) and lend support to the hypothesis that oxidative stress and subsequent AGE generation may also be involved in angiotensin-dependent end-organ damage as one common final pathway.

AGEs IN THE KIDNEY

AGE accumulation in the kidney may follow quantitative trapping of AGEs from the circulation or result from either local generation of new AGEs involving preexisting proteins or pathologically decreased AGE turnover. Figure 2 gives an example of the histological distribution of AGEs in the kidney in a patient with advanced diabetic nephropathy. The process of renal trapping of AGEs has been evidenced by chronic intravenous infusion of AGE (CML)-modified rat albumin into normal rats. After 5 mo of infusion, the AGE content of the kidneys increased by 50% in treated compared with control animals and histological features similar to diabetic glomerulosclerosis, with basement membrane widening, increased glomerular tuft volume, mesangial extracellular matrix expansion, glomerulosclerosis, and albuminuria, were present (148). This mouse infusion model had been initially described by McVerry et al. (94) as producing a pseudodiabetic renal pattern. In the experiment by Vlassara et al. (148), total serum AGE concen-

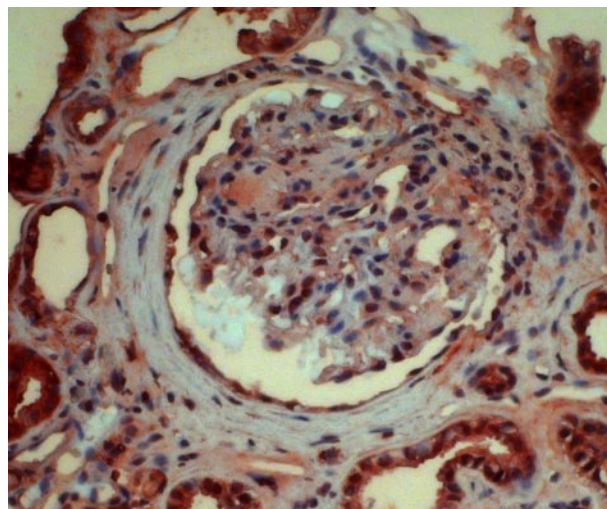


Fig. 2. AGE staining for imidazolone in a renal specimen from a patient with advanced diabetic nephropathy with glomerulosclerosis. Brown staining indicates imidazolone. Magnification: $\times 400$.

trations about twofold above those observed in human diabetes were achieved. The model, however, lacks elevated serum glucose concentrations and the typical metabolic features of diabetes. Furthermore, AGE albumin was administered to mice with normal renal function, thereby reflecting the reaction of normal kidneys to a chronic toxic stimulus. The lesions in diabetic nephropathy and chronic renal failure of other origin, however, evolve over time as a result of complex pathophysiological mechanisms, with different cellular pathways usually operative in parallel. For instance, exposure to high glucose itself may alter cellular function in the absence of AGEs. Also, the release of cytokines or activation of local homeostatic systems such as the renin-angiotensin system independently of AGE stimulation has its own established pathogenetic role (2). Nevertheless, the AGE infusion model and similar models of chronic renal injury may provide helpful insight into important pathogenetic aspects, for instance, associated with circulating AGE proteins despite their inherent limitations. Interestingly, inhibition of albumin glycation in a complementary *in vivo* model of diabetic *db/db* mice has recently been shown to ameliorate glomerular and indexes of renal end-organ damage, supporting a pivotal role of circulating AGE compounds (26, 27). In humans, renal and vascular accumulation of AGE is most prominent in diabetes mellitus, where global AGE generation occurs early and at an accelerated rate in almost any organ system. Glomerular and vascular AGE accumulation has been shown to be a major pathogenic factor precipitating diabetic vascular complications and glomerulosclerosis (72, 152).

Diabetic nephropathy is characterized by glomerular and tubular basement membrane thickening, mesangial extracellular matrix expansion, microvascular damage, and fibrotic changes in the tubulointerstitium. Almost all renal structures are susceptible to accumulate AGEs including basement membranes, mesangial and endothelial cells, podocytes, and tubules (46). Horie and colleagues (53) examined in detail the immunohistochemical localization of glycoxidation products in glomerular lesions and in the vasculature of patients with early compared with advanced diabetic nephropathy. They found enhanced CML accumulation in the expanded mesangial matrix, thickened glomerular capillary wall of early nodular lesions, and also in arterial walls of advanced diabetic nephropathy but not in normal kidneys. Niwa et al. (100) showed similar results for imidazolone. This observation could also be replicated experimentally in genetically or streptozotocin-treated diabetic rats (65, 153). Nondiabetic nephropathies, however, may display different patterns of local AGE distribution. Kidney sections from patients with IgA nephropathy, for instance, do not show glomerular CML accumulation or staining for acrolein. Rather, the glomerular matrix lesions stained positive for reactive lipoxidation products such as malondialdehyde-lysine, implicating fundamental pathophysiological differences (138). Another study by Tanji et al. (141) of renal tissue specimens from patients with diabetic nephropathy, primary and secondary focal segmental glomerulosclerosis type, hypertensive nephropathy, and lupus nephritis showed significant qualitative and quantitative differences in the distribution of CML and pentosidine, respectively, demonstrating an independent influence of the disease mechanism and the course of the disease on the type of AGE modification and the distribution of AGE-modified structures.

RECEPTOR-MEDIATED EFFECTS ON CELLULAR FUNCTION

The presence of AGE-modified proteins in tissues, the biological effects associated with their accumulation, and pharmacological inhibition studies have stimulated intensive research to identify cellular surface molecules that recognize AGEs and may induce specific cellular responses. In 1992, Schmidt and her colleagues (98, 119) first discovered a cellular surface receptor that binds AGE-modified proteins with high affinity, which was subsequently termed RAGE. Continuous research has meanwhile identified several other receptors and cell surface molecules capable of binding AGE-modified proteins. These receptors include macrophage scavenger receptors (MSR) type A and B₁ (CD36), oligosaccharyl transferase-48, termed AGE receptor 1 (AGE-R1), 80K-H phosphoprotein (AGE-R2), and galectin-3 (AGE-R3) (67, 90, 103).

Among these AGE-binding receptors, RAGE has been best characterized. It is a 35-kDa protein belonging to the immunoglobulin superfamily. Accordingly, its gene has been located on chromosome 6, between the genes for major histocompatibility complex II and III (120, 134). Cloning experiments and molecular characterization have revealed its three-dimensional structure and elucidated its functional properties (Fig. 3). RAGE is a transmembrane receptor consisting of 394 amino acids with a single hydrophobic transmembrane domain of 19 amino acids and a COOH-terminal cytosolic tail of 43 amino acids and shares homology with MUC 18, NCAM, and, partly, CD20, a B-cell activation marker (98). The extracellular part consists of a terminal V-type and two distinct C-type domains (V-C-C'), where V domains bind ligands and the highly charged cytosolic tail would mediate activation of intracellular signal transduction pathways. Because of its short cytosolic tail without apparent enzymatic activity, it has been speculated that the receptor may associate into a multimeric cell surface complex on activation before triggering cellular events involving other costimulatory proteins (160). Various viable mRNA splice variants have been detected encoding for truncated proteins with different biological properties. RAGE lacking the cytosolic tail remains anchored within the cell membrane and binds extracellular ligands without intrinsic effects but can suppress cellular signaling by full-length RAGE in a dominant-

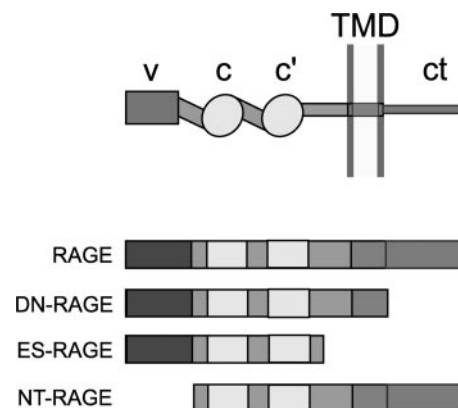


Fig. 3. Structure of the receptor for advanced glycation end products (RAGE) and major splice variants. The molecule has 3 extracellular domains (V-C-C') where the V-domain interacts with ligands. TMD, transmembrane domain; ct, cytosolic tail; DN-RAGE, dominant-negative RAGE; ES-RAGE, endogenous soluble RAGE; NT-RAGE, N-truncated RAGE.

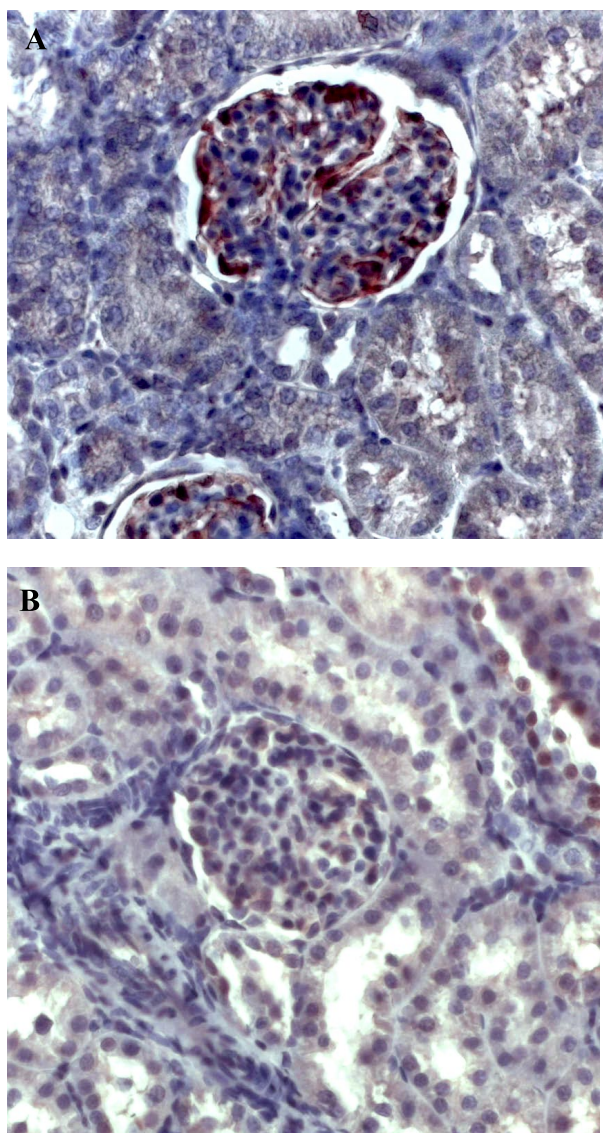


Fig. 4. Glomeruli from transgenic rats with ANG II-dependent hypertension (A) and nontransgenic controls stained immunohistochemically for CML (B). Note the positive staining for AGE in transgenic glomeruli along the outer capillary loops (brown stain). Magnification: $\times 400$.

negative way (DN-RAGE). Furthermore, endothelial cells were shown to translate a truncated RAGE variant lacking the total COOH-terminal transmembrane domain that is efficiently secreted in a paracrine way (ES-RAGE). ES-RAGE may bind to extracellular ligands independently of direct cell contact, thereby possibly preventing epitope recognition by full-length receptors located in the cell membrane. Still another RAGE isoform lacks only the small 19-amino acid transmembrane domain serving as an anchor sequence and therefore is similarly secreted. Finally, a version lacking the NH₂-terminal V-domain has been found that resides in the cell membrane but does not bind ligands with a still unclear role (73, 167).

Physiological expression of RAGE has been detected in a variety of tissues. The receptor is found on endothelial cells, vascular smooth muscle cells, peripheral blood mononuclear cells, and macrophages but also in neural tissues such as neurons, microglia, astrocytes, and peripheral nerves, the

lungs, and skeletal muscle (19, 56, 129, 130). In the human kidney, physiological RAGE expression of RAGE protein was found only on podocytes at a very low concentration. Its expression, however, could not be detected in other glomerular cell types. In contrast, tubular epithelia may stain positive for RAGE (130, 141).

The receptor has been shown to bind different AGE adducts nonspecifically. Among these, CML and pentosidine adducts are high-affinity ligands (62). RAGE therefore may bind and recognize different proteins alike, independently of their individual biological function, which suggests a biological role as a nonspecific environmental sensor. In this context, RAGE can be characterized as a pattern- or common motif-recognizing receptor with homeostatic functions accepting diverse epitopes independently of their location and mode of presentation, much like other members of its superfamily. Therefore, amyloid- β protein is similarly recognized as are other proteins, for instance, amylin, serum amyloid A, albumin, prion-derived peptides, and β_2 -microglobulin, after glycation and conformational rearrangement of their three-dimensional structure (15, 79, 164). The receptor's role, however, is not restricted to the pathological situation of AGE modification of proteins. Rather, this may be an extended ability based on its natural function as a modulator of inflammatory processes. Recent research has identified S100/calgranulins as unexpected new ligands with distinct pathophysiological properties on binding RAGE. The S100/calgranulins are a family of Ca²⁺-binding polypeptides with >20 yet identified members. Some members of the group including S100A8, S100A9, and S100A12 (calgranulin C) are released by stimulated phagocytes and may act as secretory cytokines. These S100 molecules have been shown to activate endothelial cells, mononuclear phagocytes, and lymphocytes on binding RAGE while inducing multiple proinflammatory responses, for instance, increased cyclooxygenase (COX)-2 expression (52, 111, 122). Stimulation of RAGE by S100P can directly induce cellular proliferation (8). Using a mouse model of peritonitis, administration of soluble RAGE and investigating diabetic and RAGE knockout mice, Chavakis and colleagues (24) showed that leukocyte adhesion and extravasation were tightly controlled by RAGE. The effect was independent of ICAM-1, the principal ligand for leukocyte adhesion (24). These evolving new features characterize RAGE as a versatile multiligand receptor intrinsically involved in amplifying and maintaining inflammatory responses as well as modulating cellular function and growth in response to a variety of extracellular stimuli or environmental inflammatory cytokines.

In the presence of extracellular AGE, susceptible cells can rapidly upregulate expression of RAGE. In the kidney, this effect has been consistently demonstrated for glomerular podocytes in diabetic nephropathy. In contrast, RAGE expression in other glomerular cells is generally less inducible (130, 141, 153). Furthermore, cellular expression of RAGE can be induced by AGE ligands themselves. Its expression, however, can also increase in the absence of AGEs, for instance, during inflammatory tissue remodeling or after direct cytokine stimulation by TNF- α (6, 115, 140). These observations are readily explained by the presence of NF- κ B and SP-1 binding sites in the promoter region of the RAGE gene (68). In an animal model of amyloidosis, mononuclear phagocytes invading β_2 -amyloid-containing plaques upregulated RAGE expression and released TNF- α .

Table 2. Cytokines and cellular events associated with AGE or RAGE activation

VCAM-1 ↑	Endothelial cells
ICAM-1 ↑	Endothelial cells
E-selectin ↑	Endothelial cells
PDGF ↑	Pancreatic cancer cells
eNOS ↓	Endothelial cells
Tissue factor ↑	Endothelial cells
TGF-β ↑	Mesangial cells, proximal tubular cells, vascular smooth muscle cells, macrophages
TNF-α ↑	Endothelial cells, mesangial cells, mononuclear macrophages
IGF-1 ↑	Mesangial cells
MCP-1 ↑	Mesangial cells, endothelial cells
CTGF ↑	Fibroblasts, mesangial cells
IL-6 ↑	Endothelial cells
PAI-1 ↑	Endothelial cells
RAGE ↑	Mesangial cells, endothelial cells, podocytes
VEGF ↑	Podocytes, endothelial cells, mesangial cells
ANG II-dependent cell activation ↑	Vascular smooth muscle cells
Type IV collagen expression ↑	Mesangial cells
Fibronectin ↑	Mesangial cells
Cell cycle progression ↓	Fibroblasts, mesangial cells

eNOS, endothelial nitric oxide synthase; TGF-β, transforming growth factor-β; MCP-1, monocyte chemoattractant protein-1; CTGF, connective tissue growth factor; PAI-1, plasminogen activator inhibitor-1.

Activation of RAGE finally triggers multiple intracellular signal transduction cascades depending on the individual cell type. Interaction of AGEs with RAGE, their cellular receptor, may result in enhanced intracellular oxidant stress and activation of NF-κB by redox-sensitive signaling pathways (163). AGEs may also inhibit cellular NO production, which is mediated, at least in part, by downregulation of NO synthase and increased NAD(P)H oxidase expression linking RAGE to chronic endothelial cell dysfunction (17, 110, 150). RAGE-triggered activation of NF-κB may confer a susceptible state by upregulation of RAGE itself via its promoter's own NF-κB binding site, thereby enhancing cellular capacity to bind AGEs. RAGE activation was shown to stimulate RAGE mRNA transcription directly and thus may initiate an autoamplifying loop during cell activation (68, 140). As a consequence, cells activated by RAGE may release a host of cytokines and growth factors to recruit other adjacent cells into the inflammatory reaction. The receptor's cellular actions are summarized in Table 2. RAGE activation was finally shown to induce intracellular generation of hydrogen peroxide, which was dependent on the functional integrity of NADPH oxidase. The signaling cascade involves p21^{ras}, p38, protein kinase C, and mitogen-activated protein kinases (MAPK) including Erk 1/2 (p44/p42) (25, 64, 150, 166). In mesangial fibroblasts, RAGE-mediated signals have been shown to converge also on STAT5 and p21^{waf} to control the cell cycle (21).

AGE-RAGE AXIS IN DIABETIC NEPHROPATHY

In the diabetic kidney, progressive tissue damage is closely related to the local deposition of AGEs and its pathophysiological consequences. The mechanisms leading to chronic diabetic nephropathy are complex, however, involving the renin-angiotensin system as well as many other proinflammatory cytokines and signaling pathways in parallel (121). We

shall focus here on the contributions of the AGE-RAGE axis to this process. A main feature of diabetic glomerulosclerosis is excess accumulation of extracellular matrix leading to mesangial matrix expansion as well as glomerular basement membrane thickening, which then becomes targeted by AGE modification. At a cellular level, release of TGF-β is the main trigger of this process (172). Early in the course of the disease, mesangial cells may undergo a phase of limited proliferation, but then they typically arrest in the G₁ phase of the cell cycle to produce extracellular matrix (156). In an animal model, these cellular and molecular events typical for progressive diabetic nephropathy can be partially mimicked, for instance, by chronic intravenous infusion of CML-albumin (148, 165). Furthermore, mesangial cells exposed to AGE-albumin at concentrations comparable to those found in human pathology showed increased collagen IV and TGF-β expression as well as activation of protein kinase C (28, 29, 118). Yamagishi and colleagues (161) showed that AGEs stimulated upregulation of p53 and Bax by mesangial cells in vitro, thereby facilitating apoptotic cell death. The mesangial cells also produced VEGF and monocyte chemoattractant protein-1, which stimulated prostacyclin production by cocultured endothelial cells. This pathophysiological sequence may serve as a model for the events leading to chronic glomerular injury in the diabetic kidney in vivo.

Interestingly, cultured glomerular endothelial cells were similarly shown to release TGF-β after exposure to glycated albumin, which may conversely lead to paracrine stimulation and recruitment of adjacent mesangial cells (20, 25, 157, 173). Endothelial cells exposed to AGEs may rapidly show signs of enhanced cellular oxidant stress (163). AGE-albumin or CML can also induce connective tissue factor growth factor (CTGF) expression in mesangial cells, a cytokine that stimulates extracellular matrix synthesis and functions as an important downstream mediator of TGF-β but may occur without it (144, 170). Mesangial cells also express inducible macrophage scavenger receptor, which binds AGEs independently from RAGE and thereby possibly facilitates transformation of mesangial cells into foam cells (112). Geoffroy et al. (43) described in vitro proliferation of mesangial cells exposed to AGEs and defined a role for intracellular ceramidase and sphingolipids to control this effect. Finally, inhibition of store-operated Ca²⁺ influxes by AGE-modified bovine serum albumin has been described in mesangial cells that may explain their abnormal contractile properties observed in diabetes (76).

Albuminuria is a prominent feature of progressive diabetic glomerular damage. Vascular permeability and proteinuria have been directly associated with VEGF and its dimeric transmembrane tyrosine kinase receptor (VEGFR). VEGF may induce increased endothelial fenestration, decreased molecular selectivity, and plasma exudation at the endothelial vascular border. VEGF's principal physiological expression site is the podocyte, whereas its receptor (VEGFR-2) is predominantly expressed on glomerular endothelium. In diabetic nephropathy, renal expression of RAGE increases considerably together with the expression of VEGF and VEGFR-2, which parallels glomerular capillary AGE deposition and AGE modification of the capillary basement membrane. In particular, podocytes upregulate their RAGE and VEGF expression in the chronic course of diabetic nephropathy (30, 61, 126). In vitro podocyte upregulation of RAGE expression can be observed, for instance, after

stimulation with calgranulin S100B. Treatment of diabetic rats with antibodies against VEGFR-2 and infusion of soluble RAGE (sRAGE) into diabetic *db/db* mice significantly reduced albuminuria and glomerular hypertrophy. In addition, chronic treatment of diabetic mice with sRAGE prevented abnormal renal endothelial permeability. On the other hand, homozygous RAGE null mice rendered diabetic did not develop increased renal VEGF antigen and mRNA expression as well as renal TGF- β mRNA expression compared with their nondiabetic counterparts, indicating a pivotal role for RAGE and VEGF in this process (35, 153).

After being filtered by the glomerulus, AGE peptides can be readily absorbed by proximal tubular cells. Tubular cells are sites of RAGE expression that may be actively involved in cellular AGE uptake such as megalin, another tubular receptor. In diabetic nephropathy with proteinuria, proximal tubular cells were shown to display increased NF- κ B activation. This effect could be suppressed experimentally by simultaneous administration of soluble RAGE (80, 92, 114). Similarly, after exposure to an AGE-rich extracellular milieu, cultivated tubular cells showed upregulation of TGF- β mRNA expression, which was mediated by intracellular release of ROS (159, 161). These observations were recently extended by the discovery of TGF- β -independent tubular epithelial myofibroblast transdifferentiation initiated directly by RAGE activation (69). This process may be important for the development of tubular atrophy and interstitial fibrosis during diabetic nephropathy.

Although evidence is now growing that RAGE is a key pathogenic factor involved in endothelial cell activation, vascular wall remodeling, and neointimal expansion in diabetic vascular disease as well as in nondiabetic atherosclerosis and arterial plaque formation, relatively little is known about RAGE and its role in glomerular endothelial cells (11). RAGE expression by glomerular endothelial cells was found only under pathological conditions (1). RAGE can trigger endothelial cells to produce interleukin-6, monocyte chemoattractant protein-1, induce expression of adhesion molecules, and cause intracellular generation of ROS partly by activation of NADPH oxidase with a concomitant decrease in endothelial NO synthase expression. Because endothelial cells are easily accessible to AGE-modified plasma proteins, local AGE deposition or modification of resident matrix proteins is not an obligatory prerequisite to trigger these events (110, 151, 171). At a cellular level, a synergistic effect of RAGE on ANG II-dependent activation of vascular smooth muscle cells has been described (84). Interestingly, acute hyperglycemia for as short as 2 h in humans produced by a glucose-clamping technique was capable of inducing intracellular generation of CML and activating NF- κ B in peripheral blood mononuclear cells (118). Such concomitant effects may be important steps during endothelial cell activation in the presence of AGEs.

Among the many other AGE-binding proteins, the pathophysiological importance of AGE-R3 (galectin-3) and its congeners AGE-R1 and AGE-R2 is now increasingly recognized. Galectin-3 has been deleted in a knockout mouse model. In contrast to similar RAGE knockout animals, these mice spontaneously develop glomerulosclerosis with increased proteinuria on aging (109). Functionally, galectin-3 interacts closely with AGE-R1 and AGE-R2 within a molecular aggregate on cellular surfaces termed the "AGE receptor complex." The biology of this receptor complex is not yet completely under-

stood. For instance, cultured endothelial cells readily upregulate AGE-R2 and -R3 on exposure to AGE proteins (127). In an experiment with diabetic rat kidneys, however, galectin-3 (AGE-R3) expression started to increase only about 2 mo after induction of diabetes and later, during the course of the disease, galectin-3 is involved in endocytotic activity and may actively remove AGE proteins or AGE-modified LDL. The molecule obviously exerts protective cellular effects with an aim to modulate or to contain AGE-dependent inflammatory or noxious reactions (57, 147). This notion is supported by a recent report demonstrating a negative regulatory effect of AGE-R1 on AGE-dependent proinflammatory responses in mesangial cells (71). The AGE receptors are differentially regulated during diabetic kidney disease. In contrast to RAGE, AGE-R1 was found to be downregulated, thereby decreasing its overall cytoprotective potential (50). Together, these results point to an important synergism of this group of alternative AGE binding proteins with an aim to counteract or modulate the deleterious effects of the AGE-RAGE interaction.

GENETIC ASPECTS OF RAGE

A variety of polymorphisms of the human RAGE gene have been identified and studied for their impact on disease development. The gene proved to be highly variable, bearing many single nucleotide polymorphisms with potential phenotypic effects. Hudson et al. (54, 55) described a -429T/C in the gene's promoter region and presented data suggesting an association of the -429C allele with diabetic retinopathy in type 2 diabetes with macrovascular disease. Recently, Rudofsky and co-workers (113) reported that a 63-bp deletion in the promoter of RAGE correlates with a decreased risk for nephropathy in patients with type 2 diabetes, but not in individuals with type

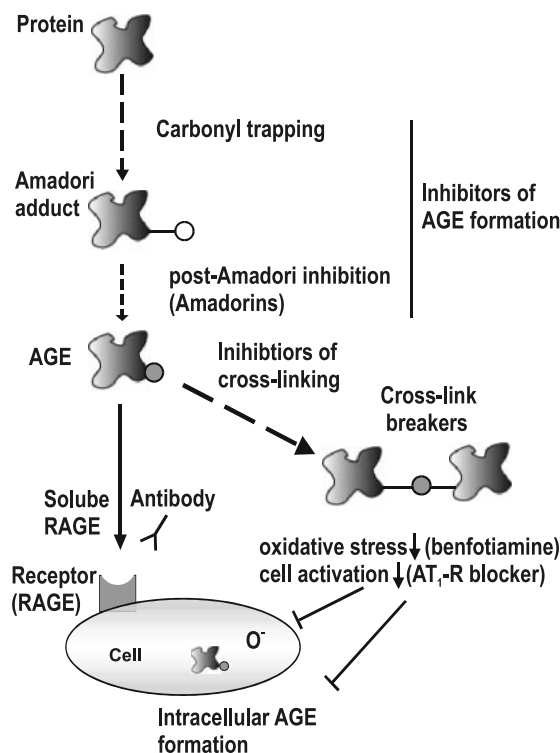


Fig. 5. Major pharmacological strategies to inhibit AGE formation or to reduce AGE-dependent end-organ damage. AT₁R, angiotensin type 1 receptor.

Table 3. Investigational compounds used to prevent AGE formation or accumulation and to reduce AGE-associated end-organ damage

Class	Compound	Mode of Action	Animal Study(ies)	Clinical Study
Inhibitor of AGE (ALE) formation	Aminoguanidine (Pimagedine)	Nucleophilic agent; reaction with glucose-derived intermediates, e.g., α,β -dicarbonyl compounds (143)	Renal profibrotic cytokines \downarrow , collagen IV \downarrow ; protein carbonylation \downarrow , albuminuria \downarrow , prevents activation of protein kinase C; aortic stiffening \downarrow (60, 70, 106, 168)	Bolton et al. (14) Bucala et al. (17)
	Pyridoxamine (Pyridorin)	Post-Amadori reaction inhibitor (Amadorin), carbonyl trapping, chelation of catalytic metal ions, inhibits superoxide radicals (5, 58, 78, 149)	Lipid-lowering effect, inhibits progression of diabetic nephropathy, albuminuria \downarrow , cross-linking of collagen n \downarrow (4, 33)	Williams et al. (155)
	OPB-9195 ALT-946	Carbonyl trapping (86) Inhibits protein cross-linking	Renal AGE accumulation \downarrow (96) Renal AGE accumulation \downarrow (38)	Miyata et al. (86)
Cross-link breaker	Alagebrium (ALT-711)	Opens AGE-derived protein cross-links (136)	Large artery stiffness \downarrow , improved ventricular function, inhibits renal fibrosis, proteinuria \downarrow , inhibits activation of PKC- α , profibrotic cytokines \downarrow , oxidative stress \downarrow , cellular transdifferentiation \downarrow , renal AGE content \downarrow (40, 65, 105, 137, 142, 158)	Kass et al. (59); DIAMOND, SAPPHIRE, SILVER, SPECTRA-trials (10)
AGE neutralization	N-phenacylthiazolium bromide (PTB)	Opens AGE-derived protein cross-links	Vascular AGE accumulation \downarrow (31)	
Other	Soluble receptor of AGE, AGE-antibodies, lysozyme ACE-inhibitors, AT ₁ -receptor blocker Thiazolidinediones HMG-CoA reductase inhibitors	Inhibits biological actions Indirect, oxidative stress \downarrow Receptor of AGE expression \downarrow (74) AGE-induced angiogenesis \downarrow (104)	AGE accumulation \downarrow , AGE clearance \uparrow (27, 70, 169) Plasma and renal AGE accumulation \downarrow (39, 117)	Sebekova et al. (116)

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

I diabetes. Finally, a relationship between the A-374A polymorphism and angiographic coronary artery disease has recently been noted, with a lower frequency of the A allele in affected compared with disease-free subjects (37).

PHARMACOLOGICAL INTERVENTIONS

A variety of pharmacological compounds and strategies have been studied in vitro as well as in vivo for their potential to prevent AGE formation or local AGE accumulation. A detailed analysis of the current literature is provided by recent topical reviews (10, 75, 154). These drugs can be divided into different classes according to their mechanism of action (Fig. 5). For instance, substances may reduce the amount of already formed AGEs by chemically reopening AGE-mediated cross-links between proteins, thereby reducing or neutralizing established end-organ damage by AGEs in the vasculature or kidney. Alagebrium is such a new prototypic compound that displays appreciable biological activity as a cross-link breaker with possible clinical implications (14). The drug ameliorates indexes of vascular stiffness and ventricular performance and reduces renal AGE content in animal models and humans (10). Many investigational drugs such as pyridoxamine or aminoguanidine, however, aim at preventing AGE formation by trapping reactive carbonyl intermediates based on their nucleophilic potential or quench ROSr and oxidative stress. Benfotiamine similarly intervenes at a cellular level (47). This preventive strategy may be preferable to clinicians as an adjunctive therapy in the specific treatment of diabetes or

chronic renal failure, delaying progressive end-organ damage. In advanced disease, compounds reducing AGE deposition or breaking established protein cross-links may become the first choice. Finally, drugs preventing AGE-induced cellular activation, for instance, inhibitors of RAGE, could be an alternative, whenever inflammatory reactions should be controlled. Dietary options, instead, could possibly become an attractive nonpharmacological alternative (23).

Although many different compounds are currently under scrutiny and extensive in vivo testing in animal models has occurred, only a few have successfully entered clinical trials thus far. Finally, none of these compounds has yet been approved for general clinical use mostly due to unfavorable side effects or because their beneficial effects were only marginal at the doses tested compared with conventional treatment strategies. These drugs, however, allow important experimental insight into AGE-related pathophysiological mechanisms at least in animal models. Table 3 gives a selected overview of important compounds and their pharmacological effects together with examples of recent clinical trials. Other substances, for instance, 6-dimethyl-aminopyridoxamine (dmaPM) (32), 2,3-diaminophenazine (107), EXO-263 (26), tenilsetam (128), hydralazine (97), diclofenac (146), and ascorbic acid (63), have been tested experimentally as inhibitors of glycation or AGE formation with varying success. Experimentally, soluble RAGE has been used extensively as a tool to block RAGE-dependent effects in vivo and in vitro (14, 108). Finally, inhibition of the renin-angiotensin system may have some

beneficial effects. Patients with nondiabetic nephropathy treated for 2 mo with an angiotensin-converting enzyme (ACE) inhibitor showed a moderate decrease of plasma AGE concentration in parallel with improved parameters of oxidative stress. Furthermore, treatment with an angiotensin type 1-receptor antagonist prevented the modest rise of AGE concentrations in a renal ablation model (116, 117). Similarly, olmesartan significantly reduced renal pentosidine content in a hypertensive diabetic rat model (97). Interestingly, AT₁-receptor antagonists and ACE inhibitors may lower the generation of serum AGEs in a cell-free in vitro system (88).

SUMMARY AND FUTURE DIRECTIONS

Formation of AGEs is an ubiquitous slow process of protein modification and degeneration by sugar molecules and reactive intermediates of oxidant and carbonyl stress that occurs in situations where the biochemical reaction equilibrium is shifted toward covalent interaction between the two reaction partners. This effect is most prominent in diabetes mellitus and chronic renal failure characterized by uremic carbonyl stress when considerable amounts of AGE-modified proteins accumulate in plasma and tissues. In the kidney, AGEs and RAGE, their main receptor, are intrinsically involved in accelerating diabetic and uremic glomerulopathy with tubulointerstitial damage. Furthermore, nonreceptor-mediated intracellular AGE generation initiated by mitochondrial superoxide release plays an independent role in diabetes. The pathophysiological implications of AGEs, RAGE, and other AGE binding proteins are rapidly expanding, whereas their role in vascular remodeling, chronic inflammatory processes, and cellular dysfunction becomes increasingly recognized.

Despite these advances, however, many important aspects of AGEs and their role in renal biology still remain unclear. For instance, the interactions of other homeostatic systems involved in renal pathology such as the renin-angiotensin, NO, or the endothelin systems with the AGE-RAGE axis are not well understood. Studies will therefore have to dissect the relative contributions of each system to oxidative stress, AGE generation, and AGE receptor-mediated or -independent events. With potent inhibitors available, future in vivo studies should address the relevance of the many in vitro findings using also transgenic and nondiabetic animal models to further extend our knowledge beyond classic metabolic pathways. In this context, the renin-angiotensin system is a primary candidate given its clinical importance as a target for therapeutic interventions to slow the progression of chronic renal failure (42). Finally, in view of the limited effects of drug therapy or dialysis modalities on AGE accumulation in end-stage renal failure due to the longevity of established AGE proteins, particular emphasis should be put in the future on preventive pharmacological strategies and early stages of renal damage. Less toxic compounds are required, and the development of a synthetic RAGE inhibitor could be of interest. Such human studies could also include combination therapies to exploit synergistic effects.

ACKNOWLEDGMENTS

We thank Dr. M. Ruster for providing the micrograph shown in Fig. 2.

REFERENCES

1. Abel M, Ritthaler U, Zhang Y, Deng Y, Schmidt AM, Greten J, Sernau T, Wahl P, Andrassy K, and Ritz E. Expression of receptors

- for advanced glycosylated end-products in renal disease. *Nephrol Dial Transplant* 10: 1662–1667, 1995.
2. Agarwal R, Campbell RC, and Warnock DG. Oxidative stress in hypertension and chronic kidney disease: role of angiotensin II. *Semin Nephrol* 24: 101–114, 2004.
3. Ahmed N and Thornally PJ. Quantitative screening of protein biomarkers of early glycation, advanced glycation, oxidation and nitrosation in cellular and extracellular proteins by tandem mass spectrometry multiple reaction monitoring. *Biochem Soc Transact* 31: 1417–1422, 2003.
4. Alderson NL, Chachich ME, Youssef NN, Beattie RJ, Nachtigal M, and Thorpe SR, and Baynes JW. The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. *Kidney Int* 63: 2123–2133, 2003.
5. Amarnath V, Amarnath K, Amarnath K, Davies S, and Roberts LJ. Pyridoxamine: an extremely potent scavenger of 1,4-dicarbonyls. *Chem Res Toxicol* 17: 410–415, 2004.
6. Anderson MM, Hazen SL, Hsu FF, and Heinecke JW. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanol, and acrolein: a mechanism for the generation of highly reactive α -hydroxy and α,β -unsaturated aldehydes by phagocytes at sites of inflammation. *J Clin Invest* 99: 424–432, 1997.
7. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening. *J Hypertens* 21: 3–12, 2003.
8. Arumugam T, Simeone D, Schmidt AM, and Logsdon C. S100P stimulates cell proliferation and survival via receptor for advanced glycation end products (RAGE). *J Biol Chem* 279: 5059–5065, 2004.
9. Asano M, Fujita Y, Ueda Y, Suzuki D, Miyata T, Sakai H, and Saito A. Renal proximal tubular metabolism of protein-linked pentosidine, an advanced glycation end product. *Nephron* 91: 688–694, 2002.
10. Bakris GL, Bank AJ, Kass DA, Neutel JM, Preston RA, and Oparil S. Advanced glycation end-product cross-link breakers: a novel approach to cardiovascular pathologies related to the aging process. *Am J Hypertens* 17: 23S–30S, 2004.
11. Basta G, Lazzarini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, and De Caterina R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE. A mechanism for amplification of inflammatory responses. *Circulation* 105: 816–822, 2002.
12. Bishara NB, Dunlop ME, Murphy TV, Darby IA, Sharmini Rajanayagam MA, and Hill MA. Matrix protein glycation impairs agonist-induced intracellular Ca²⁺ signaling in endothelial cells. *J Cell Physiol* 193: 80–92, 2002.
13. Bohlender J, Fukamizu A, Lippoldt A, Nomura T, Dietz R, Menard J, Murakami K, Luft FC, and Ganten D. High human renin hypertension in transgenic rats. *Hypertension* 29: 428–434, 1997.
14. Boltong WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spiniowitz BS, Whittier FC, and Wuerth JP for the ACTION Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 24: 32–40, 2004.
15. Bouma B, Kroon-Batenburg LMJ, Wu YP, Brünjes B, Posthuma G, Kranenburg O, de Groot PG, Voest EE, and Gebbink MFG. Glycation induces formation of amyloid cross- β structure in albumin. *J Biol Chem* 278: 41810–41819, 2003.
16. Bucala R, Tracey KJ, and Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J Clin Invest* 87: 432–438, 1991.
17. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, and Vlassara H. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci USA* 91: 9441–9445, 1994.
18. Brancaccio D, Gallieni M, Niwa T, Braidotti P, and Coggi G. Ultrastructural localization of advanced glycation end products and β_2 -microglobulin in dialysis amyloidosis. *J Nephrol* 13: 129–136, 2000.
19. Brett J, Schmidt AM, Yan SD, Zou YS, Weidman E, Pinsky D, Nowygrod R, Neeper M, Przysiecki C, and Shaw A. Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. *Am J Pathol* 143: 1699–1712, 1993.
20. Brizzi MF, Dentelli P, Rosso A, Calvi C, Gambino R, Cassader M, Salvadio G, Deferrari G, Camussi G, Pegoraro L, Pagano G, and Callo-Perin P. RAGE- and TGF- β receptor mediated signals converge on STAT5 and p21waf to control cell-cycle progression of mesangial

- cells: a possible role in the development and progression of diabetic nephropathy. *FASEB J* 18: 1249–1251, 2004.
21. **Brownlee M, Vlassara H, Kooney A, Ulrich P, and Cerami A.** Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* 232: 1629–1632, 1986.
 22. **Busch M, Franke S, Müller A, Wolf M, Gerth J, Ott U, Niwa T, and Stein G.** Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the C-reactive protein. *Kidney Int* 66: 338–347, 2004.
 23. **Cai W, He JC, Zhu L, Peppas M, Lu C, Uribarri J, and Vlassara H.** High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. *Circulation* 110: 285–291, 2004.
 24. **Chavakis T, Bierhaus A, Al-Fakhri N, Schneider D, Witte S, Linn T, Nagashima M, Morser J, Arnold B, Preissner K, and Nawroth P.** The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J Exp Med* 198: 1507–1515, 2003.
 25. **Chen S, Cohen MP, Lautenslager GT, Shearman CW, and Ziyadeh FN.** Glycated albumin stimulates TGF- β_1 production and protein kinase C activity in glomerular endothelial cells. *Kidney Int* 59: 673–681, 2001.
 26. **Cohen MP, Masson N, Hud E, Ziyadeh F, Han DC, and Clements RS.** Inhibiting albumin glycation ameliorates diabetic nephropathy in the *db/db* mouse. *Exp Nephrol* 8: 135–143, 2000.
 27. **Cohen MP, Sharma K, Jin Y, Hud E, Wu VY, Tomaszewski J, and Ziyadeh FN.** Prevention of diabetic nephropathy in *db/db* mice with glycated albumin antagonists: a novel strategy. *J Clin Invest* 95: 2338–2345, 1995.
 28. **Cohen MP, Ziyadeh FN, Lautenslager GT, Cohen JA, and Shearman CW.** Glycated albumin stimulation of PKC- β activity is linked to increased collagen IV in mesangial cells. *Am J Physiol Renal Physiol* 276: F684–F690, 1999.
 29. **Cohen MP, Ziyadeh FN, Hong SW, Shearman CW, Hud E, Lautenslager GT, Iglesias-De La Cruz MC, and Chen S.** Inhibiting albumin glycation in vivo ameliorates glomerular overexpression of TGF- β_1 . *Kidney Int* 61: 2025–2032, 2002.
 30. **Cooper ME, Vranes D, Yousef S, Stacker SA, Cox AJ, Rizkalla B, Casley DJ, Bach LA, Kelly DJ, and Gilbert RE.** Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. *Diabetes* 48: 2229–2239, 1999.
 31. **Cooper ME, Thallas V, Forbes J, Scalbert E, Sastra S, Darby I, and Souliis T.** The cross-link breaker, *N*-phenacyl-thiazolium bromide prevents vascular advanced glycation end-product accumulation. *Diabetologia* 43: 660–664, 2000.
 32. **Culbertson SM, Vassilenko EI, Morrison LD, and Ingold KU.** Paradoxical impact of antioxidants on post-amadori glycooxidation. Counterintuitive increase in the yields of pentosidine and *N*^ε-carboxymethyllysine using a novel multifunctional pyridoxamine derivative. *J Biol Chem* 278: 38384–38394, 2003.
 33. **Degenhardt TP, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW, Thorpe SR, and Baynes JW.** Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. *Kidney Int* 61: 939–950, 2002.
 34. **Deuther-Conrad W, Franke S, Sommer M, Henle T, and Stein G.** Differences in the modulating potential of advanced glycation end product (AGE) peptides versus AGE proteins. *Kidney Int* 78: S63–S66, 2001.
 35. **De Vriese AS, Tiltin RG, Elger M, Stephan CC, Kriz W, and Lameire NH.** Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol* 12: 993–1000, 2001.
 36. **Dyer DG, Dun JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, and Baynes JW.** Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91: 2463–2469, 1993.
 37. **Falcone C, Campo I, Emanuele E, Buzzi MP, Zorzetto M, Sbarsi I, and Cuccia M.** Relationship between the -374T/A RAGE gene polymorphism and angiographic coronary artery disease. *Int J Mol Med* 14: 1061–1064, 2004.
 38. **Forbes JM, Souliis T, Thallas V, Panagiotopoulos S, Long DM, Vasan S, Wagle D, Jerums G, and Cooper ME.** Renoprotective effects of a novel inhibitor of advanced glycation. *Diabetologia* 44: 108–114, 2001.
 39. **Forbes JM, Thomas MC, Thorpe SR, Alderson NL, and Cooper ME.** The effects of valsartan on the accumulation of circulating and renal advanced glycation end products in experimental diabetes. *Kidney Int* 66, Suppl 92: S105–S107, 2004.
 40. **Forbes JM, Thallas V, Thomas MC, Founds HW, Burns WC, Jerums G, and Cooper ME.** The breakdown of preexisting advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes. *FASEB J* 17: 1762–1764, 2003.
 41. **Franke S, Müller A, Sommer M, Busch M, Kientsch-Engel R, and Stein G.** Serum levels of total homocysteine, homocysteine metabolites and of advanced glycation end products (AGEs) in patients after renal transplantation. *Clin Nephrol* 59: 88–97, 2003.
 42. **Fukami K, Ueda S, Yamagishi SI, Kato S, Inagaki Y, Takeuchi M, Motomiya Y, Bucala R, Iida S, Makai K, Imaizumi T, Cooper ME, and Okuda S.** AGEs activate mesangial TGF- β -Smad signaling via an angiotensin II type I receptor interaction. *Kidney Int* 66: 2137–2147, 2004.
 43. **Geoffroy K, Wiernsperger N, Lagarde M, and El Bawab S.** Bimodal effects of advanced glycation end products on mesangial cell proliferation is mediated by neutral ceramidase regulation and endogenous sphingolipids. *J Biol Chem* 279: 34343–34352, 2004.
 44. **Giardino I, Edelstein D, and Brownlee M.** Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters fibroblast growth factor activity: a model for intracellular glycosylation in diabetes. *J Clin Invest* 94: 110–117, 1994.
 45. **Giardino I, Edelstein D, and Brownlee M.** BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. *J Clin Invest* 97: 1422–1428, 1996.
 46. **Gugliucci A and Bendayan M.** Reaction of advanced glycation end-products with renal tissue from normal and streptozotocin-induced diabetic rats. An ultrastructural study using colloidal gold cytochemistry. *J Histochem Cytochem* 43: 591–600, 1995.
 47. **Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, and Brownlee M.** Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9: 294–299, 2003.
 48. **Hein G, Wiegand R, Lehmann G, Stein G, and Franke S.** Advanced glycation end-products pentosidine and *N*^ε-carboxymethyllysine are elevated in serum of patients with osteoporosis. *Rheumatology* 42: 1242–1246, 2003.
 49. **Haitoglu CS, Tsilbary EC, Brownlee M, and Charonis AS.** Altered cellular interactions between endothelial cells and nonenzymatically glycosylated laminin/type IV collagen. *J Biol Chem* 267: 12404–12407, 1992.
 50. **He CJ, Zheng F, Stitt A, Striker L, Hattori M, and Vlassara H.** Differential expression of renal AGE receptor genes in NOD mice: possible role in nonobese diabetic renal disease. *Kidney Int* 58: 1931–1940, 2000.
 51. **Henle T, Deppech R, Beck W, Hergesell O, Hänsch GM, and Ritz E.** Advanced glycated end-products (AGE) during haemodialysis treatment: discrepant results with different methodologies reflecting the heterogeneity of AGE compounds. *Nephrol Dial Transplant* 14: 1968–1975, 1999.
 52. **Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, and Schmidt AM.** RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97: 889–901, 1999.
 53. **Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersele de Strihou C, Monnier VM, Witztum JL, and Kurokawa K.** Immunohistochemical colocalization of glycooxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycooxidative stress in the pathogenesis of diabetic nephropathy. *J Clin Invest* 100: 2995–3004, 1997.
 54. **Hudson BI, Stickland MH, and Grant PJ.** Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene: prevalence in type 2 diabetes and ethnic groups. *Diabetes* 47: 1155–1157, 1998.
 55. **Hudson BI, Stickland MH, Futers TS, and Grant PJ.** Study of -429T/C and 374T/A receptor for advanced glycation end products promoter polymorphisms in diabetic and nondiabetic subjects with macrovascular disease. *Diabetes Care* 24: 2004, 2001.
 56. **Huttenen HJ, Fages C, and Rauvala H.** Receptor for advanced glycation end-products (RAGE)-mediated neurite outgrowth and activation

- of NF- κ B require the cytoplasmic domain of the receptor but different downstream signalling pathways. *J Biol Chem* 274: 19919–19924, 1999.
57. **Iacobini C, Amadio L, Oddi G, Ricci C, Barsotti P, Missori S, Sorcini M, Di Mario U, Pricci F, and Pugliese G.** Role of galectin-3 in diabetic nephropathy. *J Am Soc Nephrol* 14: S264–S270, 2003.
 58. **Jain SK and Lim G.** Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycosylation, and (Na⁺-K⁺)-ATPase activity reduction in high glucose-treated human erythrocytes. *Free Rad Biol Med* 30: 232–237, 2001.
 59. **Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, DeGroof RC, and Lakatta EG.** Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 104: 1464–1470, 2001.
 60. **Kelly D, Gilbert RE, Cox AJ, Soulis T, Jerums G, and Cooper ME.** Aminoguanidine ameliorates overexpression of pro-sclerotic growth factors and collagen deposition in experimental diabetic nephropathy. *J Am Soc Nephrol* 12: 2098–2107, 2001.
 61. **Khamaisi M, Schrijvers BF, De Vriese AS, Raz I, and Flyvbjerg A.** The emerging role of VEGF in diabetic kidney disease. *Nephrol Dial Transplant* 18: 1427–1430, 2003.
 62. **Kislinger T, Fu C, Huberi B, Qu W, Taguchi A, Yan S, Hofmann M, Yan S, Pischetsrieder M, Stern D, and Schmidt AM.** N^ε-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem* 274: 31740–31749, 1999.
 63. **Krone CA and Ely JT.** Ascorbic acid, glycation, glycohemoglobin and aging. *Med Hypotheses* 62: 275–279, 2004.
 64. **Lander MM, Tauras JM, Ogiste JS, Hori O, Moss RA, and Schmidt AM.** Activation of the receptor of advanced glycation end products triggers a p21ras-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem* 272: 17810–17814, 1997.
 65. **Lassila M, Seah KK, Allen TJ, Thallas V, Thomas MC, Candido R, Burns WC, Forbes JM, Calkin AC, Cooper ME, and Jandeleit-Dahm KA.** Accelerated nephropathy in diabetic apolipoprotein e-knockout mouse: role of advanced glycation end products. *J Am Soc Nephrol* 15: 2125–2138, 2004.
 66. **Leslie RDG, Beyan H, Sawtell P, Boehm BO, Spector TD, and Snieder H.** Level of an advanced glycated end product is genetically determined. A study of normal twins. *Diabetes* 52: 2441–2444, 2003.
 67. **Li YM, Mitsuhashi T, Wojciechowski D, Shimizu N, Li J, Stitt A, He C, Banerjee D, and Vlassara H.** Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. *Proc Natl Acad Sci U S A* 93: 11047–11052, 1996.
 68. **Li J and Schmidt AM.** Characterization and functional analysis of the promoter of RAGE. *J Biol Chem* 272: 16498–16506, 1997.
 69. **Li JH, Wang W, Huang XR, Oldfield M, Schmidt AM, Cooper ME, and Lan HY.** Advanced glycation end products induce tubular epithelial-myofibroblast transition through the RAGE-ERK1/2 MAP kinase signaling pathway. *Am J Pathol* 164: 1389–1397, 2004.
 70. **Lin YT, Tseng YZ, and Chang KC.** Aminoguanidine prevents fructose-induced arterial stiffening in Wistar rats: aortic impedance analysis. *Exp Biol Med* 229: 1038–1045, 2004.
 71. **Lu C, He JC, Liu H, Zhu L, and Vlassara H.** Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. *Proc Natl Acad Sci USA* 101: 11767–11772, 2004.
 72. **Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, and Vlassara H.** Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 325: 836–842, 1991.
 73. **Malherbe P, Richards JG, Thompson A, Diener C, Schuler A, and Huber G.** cDNA cloning of a novel secreted isoform of the human receptor for advanced glycation end products and characterisation of cells co-expressing cell-surface scavenger receptors and Swedish mutant amyloid precursor protein. *Mol Brain Res* 71: 159–170, 1999.
 74. **Marx N, Walcher D, Ivanova N, Rautzenberg K, Jung A, Friedl R, Hombach V, de Caterina R, Basta G, Wautier MP, and Wautiers JL.** Thiazolidinediones reduce endothelial expression of receptors of advanced glycation end products. *Diabetes* 53: 2662–2668, 2004.
 75. **Mene P, Festuccia F, and Pugliese F.** Clinical potential of advanced glycation end-product inhibitors in diabetes mellitus. *Am J Cardiovasc Drugs* 3: 315–320, 2003.
 76. **Mene P, Pascale C, Teti A, Bernardini S, Cinotti GA, and Pugliese F.** Effects of advanced glycation end products on cytosolic Ca²⁺ signaling of cultured human mesangial cells. *J Am Soc Nephrol* 10: 1478–1486, 1999.
 77. **Metz TO, Alderson NL, Chachich M, Thorpe SR, and Baynes JW.** Pyridoxamine traps intermediates in lipid peroxidation reactions in vivo. Evidence on the role of lipids in chemical modification of protein and development of diabetic complications. *J Biol Chem* 278: 42012–42019, 2003.
 78. **Metz TO, Alderson NL, Thorpe SR, and Baynes JW.** Pyridoxamine, an inhibitor of advanced glycation and lipoxidation reactions: a novel therapy for treatment of diabetic complications. *Arch Biochem Biophys* 419: 41–49, 2003.
 79. **Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, and Schmidt AM.** The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE- β_2 -microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. *J Clin Invest* 98: 1088–1094, 1996.
 80. **Miyata T, Ueda Y, Horie K, Nankagu M, Tanaka S, van Ypersele de Strihou C, and Kurokawa K.** Renal catabolism of advanced glycation end products: the fate of pentosidine. *Kidney Int* 53: 416–422, 1998.
 81. **Miyata T, Ueda Y, Shinzato T, Iida Y, Tanaka S, Kurokawa K, van Ypersele de Strihou C, and Maeda K.** Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 7: 1198–1206, 1996.
 82. **Miyata T, Ueda Y, Yamada Y, Saito A, Jadoul M, van Ypersele de Strihou C, and Kurokawa K.** Carbonyl stress in uremia: accumulation of carbonyls accelerate the formation of pentosidine, an advanced glycation end product. *J Am Soc Nephrol* 9: 2349–2356, 1998.
 83. **Miyata T, Ueda Y, Yoshida A, Sugiyama S, Iida Y, Jadoul M, Maeda K, Kurokawa K, and van Ypersele de Strihou C.** Clearance of pentosidine, an advanced glycation end product, by different modalities of renal replacement therapy. *Kidney Int* 51: 880–887, 1997.
 84. **Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, and Baynes JW.** Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 54: 1290–1295, 1998.
 85. **Miyata T, Kurokawa K, and van Ypersele de Strihou C.** Advanced glycation and lipoxidation end products: role of reactive carbonyl compounds generated during carbohydrate and lipid metabolism. *J Am Soc Nephrol* 11: 1744–1752, 2000.
 86. **Miyata T, Ueda Y, Asahi K, Izuhara Y, Inagi R, Saito A, van Ypersele de Strihou C, and Kurokawa K.** Mechanism of the inhibitory effect of OPB-9195 [(6)-2-Isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide] on advanced glycation end product and advanced lipoxidation end product formation. *J Am Soc Nephrol* 11: 1719–1725, 2000.
 87. **Miyata T, van Ypersele de Strihou C, Kurokawa K, and Baynes JW.** Alterations in non-enzymatic biochemistry in uremia: origin and significance of “carbonyl stress” in long-term uremic complications. *Kidney Int* 55: 389–399, 1999.
 88. **Miyata T, van Ypersele de Strihou C, Ueda Y, Ichimori K, Inagi R, Onogi H, Ishikawa N, Nangaku M, and Kurokawa K.** Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in-vitro the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 13: 2478–2487, 2002.
 89. **Miyata T, Wada Y, Cai Z, Iida Y, Horie K, Yasuda Y, Maeda K, Kurokawa K, and van Ypersele de Strihou C.** Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 51: 1170–1181, 1997.
 90. **Miyazaki A, Nakamura H, and Horiuchi S.** Scavenger receptors that recognize advanced glycation end products. *Trends Cardiovasc Med* 12: 258–262, 2002.
 91. **Mizutani K, Ikeda K, Kawai Y, and Yamori Y.** Biochemical properties and chemical composition of the aorta in genetic hypertensive rats. *J Hypertens* 17: 481–487, 1999.
 92. **Morcos M, Sayed AAR, Bierhaus A, Yard B, Waldherr R, Merz W, Kloeting I, Schleicher E, Mentz S, Abd el Baki RF, Tritsler H, Kasper M, Schwenger V, Hamann A, Dugi KA, Schmidt AM, Stern D, Ziegler R, Haering HU, Andrassy M, Van der Woude F, and Nawroth PP.** Activation of tubular epithelial cells in diabetic nephropathy. *Diabetes* 51: 3532–3544, 2002.

93. Mott JD, Khalifah RG, Nagase H, Shield CF III, Hudson JK, and Hudson BG. Nonenzymatic glycation of type IV collagen and matrix metalloproteinase susceptibility. *Kidney Int* 52: 1302–1312, 1997.
94. McVerry BA, Fisher C, Hopp A, and Huehns ER. Production of pseudodiabetic renal glomerular changes in mice after repeated injections of glucosylated proteins. *Lancet* 1: 738–740, 1980.
95. Nagai R, Unno Y, Hayashi MC, Masuda S, Hayase F, Kinane N, and Horiuchi S. Peroxynitrite induces formation of N^ε-(carboxymethyl) lysine by cleavage of amadori product and generation of glucosone and glyoxal from glucose. Novel pathways for protein modification by peroxynitrite. *Diabetes* 51: 2833–2839, 2002.
96. Nakamura S, Tachikawa T, Tobita K, Aoyama I, Takayama F, Enomoto A, and Niwa T. An inhibitor of advanced glycation end product formation reduces N^ε-(carboxymethyl)lysine accumulation in glomeruli of diabetic rats. *Am J Kidney Dis* 41: S68–S71, 2003.
97. Nangaku M, Miyata Sada T, Mizuno M, Inagi R, Ueda Y, Ishikawa N, Yuzawa H, Koike H, van Ypersele de Strihou C, and Kurokawa K. Anti-hypertensive agents inhibit in vivo the formation of advanced glycation end products and improve renal damage in a type 2 diabetic nephropathy rat model. *J Am Soc Nephrol* 14: 1212–1222, 2003.
98. Nepper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern DM, and Shaw A. Cloning and expression of RAGE: a cell surface receptor for advanced glycation end products of proteins. *J Biol Chem* 267: 14988–15004, 1992.
99. Nishikawa T, Edelstein D, Du XL, Yamagishi SI, Matsumura T, Kaneda Y, Yorek MA, David Beebek Oates PJ, Hammes HP, Giardino I, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
100. Niwa T, Katsuzaki T, Miyazaki S, Miyazaki T, Ishizaki Y, Hayase F, Tatemichi N, and Takei Y. Immunohistochemical detection of imidazolone, a novel advanced glycation end product, in kidneys and aortas of diabetic patients. *J Clin Invest* 99: 1272–1280, 1997.
101. Niwa T, Katsuzaki T, Momoi T, Miyazaki T, Ogawa H, Saito A, Miyazaki S, Maeda K, Tatemichi N, and Takei Y. Modification of β_{2m} with advanced glycation end products as observed in dialysis related amyloidosis by 3-DG accumulating in uremic serum. *Kidney Int* 49: 861–867, 1996.
102. Odani H, Shinzato H, Matsumoto Y, Usami J, and Maeda K. Increase in three α,β -dicarbonyl compound levels in human uremic plasma: specific in vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 256: 89–93, 1999.
103. Ohgami N, Nagai R, Ikemoto M, Arai H, Miyazaki A, Hakamaata H, Horiuchi S, and Nakayama H. CD36 serves as a receptor for AGEs. *J Diabetes Compl* 16: 56–59, 2002.
104. Okamoto T, Yamagishi SI, Inagaki Y, Amano S, Koga K, Abe R, Takeuchi M, Ohno S, Yoshimura A, and Makita Z. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. *FASEB J* 16: 1928–1630, 2002.
105. Oldfield MD, Bach LA, Forbes JM, Nikolic-Paterson D, McRobert A, Thallas V, Atkins RC, Osicka T, Jerums G, and Cooper ME. Advanced glycation end products cause epithelial-myofibroblast trans-differentiation via the receptor for advanced glycation end products (RAGE). *J Clin Invest* 108: 1853–1863, 2001.
106. Osicka TM, Yu Y, Lee V, Panagiotopoulos S, Kemp BE, and Jerums G. Aminoguanidine and ramipril prevent diabetes-induced increases in protein kinase C activity in glomeruli, retina and mesenteric artery. *Clin Sci (Colch)* 100: 249–257, 2001.
107. Oturai PS, Christensen M, Rolin B, Pedersen KE, Mortensen SB, and Boel E. Effects of advanced glycation end-product inhibition and cross-link breakage in diabetic rats. *Metabolism* 49: 996–1000, 2000.
108. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ, Chow WS, Stern D, and Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 4: 1025–1031, 1998.
109. Pugliese G, Pricci F, Iacobini C, Leto G, Amadio L, Barsotti Frigeri L, Hsu DK, Vlassara H, Liu F, and Di Mario U. Accelerated diabetic glomerulopathy in galactin-3/AGE receptor 3 knockout mice. *FASEB J* 15: 2471–2479, 2001.
110. Rashid G, Benchetrit S, Fishman D, and Bernheim J. Effect of advanced glycation end-products on gene expression and synthesis of TNF- α and endothelial nitric oxide synthase by endothelial cells. *Kidney Int* 66: 1099–1106, 2004.
111. Roth J, Vogt T, Sorg C, and Sundkotter C. Phagocyte-specific S100 proteins: a novel group of proinflammatory molecules. *Trends Immunol* 24: 155–158, 2003.
112. Ruan XZ, Varghese Z, Powis SH, and Moorhead JF. Human mesangial cells express inducible macrophage scavenger receptor. *Kidney Int* 56: 440–451, 1999.
113. Rudofsky G Jr, Isermann B, Schilling T, Schiekofe S, Andrassy M, Schneider JG, Morcos M, Humpert PM, Sayed AA, Witte S, Renn W, Pfohl M, Hamann A, Nosikov V, Schleicher E, Haring HU, Rudofsky G, Ritz E, Nawroth PP, and Bierhaus A. A 63bp deletion in the promoter of rage correlates with a decreased risk for nephropathy in patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 112: 135–141, 2004.
114. Saito A, Nagai R, Tanuma A, Hama H, Cho K, Takeda T, Yoshida Y, Toda T, Shimizu F, Horiuchi S, and Gejyo F. Role of megalin in endocytosis of advanced glycation end products: implications for a novel protein binding to both megalin and advanced glycation end products. *J Am Soc Nephrol* 14: 1123–1131, 2003.
115. Sakaguchi T, Yan SF, Yan SD, Rong LL, Sousa M, Belov D, Andrassy M, Marso SP, Duda S, Arnold B, Liliensiek B, Nawroth PP, Stern DM, Schmidt AM, and Naka Y. Arterial restenosis: central role of RAGE-dependent neointimal expansion. *J Clin Invest* 111: 959–972, 2003.
116. Sebekova K, Gazdikova K, Syrova D, Blazicek P, Schinzel R, Heidland A, Spustova V, and Dzurik R. Effects of ramipril in nondiabetic nephropathy: improved parameters of oxidative stress and potential modulation of advanced glycation end products. *J Hum Hypertens* 17: 265–270, 2003.
117. Sebekova K, Schinzel R, Munch G, Krivosikova Z, Dzurik R, and Heidland A. Advanced glycation end-product levels in subtotaly nephrectomized rats. Beneficial effects of angiotensin II receptor 1 antagonist losartan. *Miner Electrolyte Metab* 25: 380–383, 1999.
118. Schiekofe S, Andrassy M, Chen J, Rudofsky G, Schneider J, Wendt T, Stefan N, Humpert P, Fritsche A, Stumvoll M, Schleicher E, Haring HU, Nawroth PP, and Bierhaus A. Acute hyperglycemia causes intracellular formation of CML and activation of ras, p42, MAPK, and nuclear factor κ B in PBMCs. *Diabetes* 52: 621–633, 2003.
119. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Sposito C, Hegarty H, Hurley W, and Claus M. Isolation and characterization of binding proteins for advanced glycosylation endproducts from lung tissue which are present on the endothelial surface. *J Biol Chem* 267: 14987–14997, 1992.
120. Schmidt AM, Yan SD, Yan SF, and Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 108: 949–955, 2001.
121. Schrijvers BF, De Vriese AS, and Flyvbjerg A. From hyperglycemia to diabetic kidney disease: the roles of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. *Endocr Rev* 25: 971–1010, 2004.
122. Shanmugam N, Kim YS, Lanting L, and Natarajan R. Regulation of cyclooxygenase-2 expression by ligation of the receptor of advanced glycation end products. *J Biol Chem* 278: 34834–34844, 2003.
123. Shaw SS, Schmidt AM, Banas AK, Wang X, Stern DM, and Marrero MB. S100B-RAGE-mediated augmentation of angiotensin II-induced activation of JAK2 in vascular smooth muscle cells is dependent on PLD2. *Diabetes* 52: 2381–2388, 2003.
124. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, and Brownlee M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J Clin Invest* 101: 1142–1147, 1998.
125. Shoda I, Miyata S, Liu BF, Yamada H, Ohara T, Suzuki K, Oimomi M, and Kasuga M. Inhibitory effects of tenilsetam on the Maillard reaction. *Endocrinology* 138: 1886–1892, 1997.
126. Simon M, Rockl W, Hornig C, Grone EF, Theis H, Weich HA, Fuchs E, Yayon A, and Grone HJ. Receptors of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in fetal and adult human kidney: localization and [¹²⁵I] VEGF binding sites. *J Am Soc Nephrol* 9: 1032–1044, 1998.
127. Sitt AW, He C, and Vlassara H. Characterization of the advanced glycation end-product receptor complex in human vascular endothelial cells. *Biochem Biophys Res Commun* 256: 549–556, 1999.
128. Singh R, Bardou A, Mori T, and Beilin L. Advanced glycation end-products: a review. *Diabetologia* 44: 129–146, 2001.

129. Sorci G, Riuzzi F, Arcuri C, Giambanco I, and Donato R. Amphoterin stimulates myogenesis and counteracts the antimyogenic factors basic fibroblast growth factor and S100B via RAGE binding. *Mol Cell Biochem* 24: 4880–4894, 2004.
130. Souliis T, Thallas V, Youssef S, Gilbert R, McWilliam B, Murray-McIntosh R, and Cooper M. Advanced glycation end products and their receptors co-localise in rat organs susceptible to diabetic microvascular injury. *Diabetologia* 40: 619–628, 1997.
131. Stein G, Busch M, Müller A, Wendt T, Franke C, Niwa T, and Franke S. Are advanced glycation end products cardiovascular risk factors in patients with CRF? *Am J Kidney Dis* 41, Suppl 1: S52–S56, 2003.
132. Stein G, Franke S, Mahiout A, Schneider S, Sperschneider H, Borst S, and Vienken J. Influence of dialysis modalities on serum AGE levels in end-stage renal disease patients. *Nephrol Dial Transplant* 16: 999–1008, 2001.
133. Stopper H, Schinzel R, Sebekova K, and Heidland A. Genotoxicity of advanced glycation end products in mammalian cells. *Cancer Lett* 190: 151–156, 2003.
134. Sugaya K, Fukagawa T, Matsumoto KI, Mita K, Takahashi EI, Ando A, Inoko H, and Ikemura T. Three genes in the human MHC class III region near the junction with class II: gene for RAGE, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene int-2. *Genomics* 23: 408–419, 1994.
135. Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S, van Ypersele de Strihou C, and Kurokawa K. Plasma level of pentosidine, an advanced glycation end product, in diabetic patients. *J Am Soc Nephrol* 9: 1681–1688, 1998.
136. Susic D, Varagic J, Ahn J, and Frohlich ED. Crosslink breakers: a new approach to cardiovascular therapy. *Curr Opin Cardiol* 19: 336–340, 2004.
137. Susic D, Varagic J, Ahn J, and Frohlich ED. Cardiovascular and renal effects of a collagen cross-link breaker (ALT-711) in adult and aged spontaneously hypertensive rats. *Am J Hypertens* 17: 328–333, 2004.
138. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, Uchida K, Izuhara Y, Yagame M, Sakai H, and Kurokawa K. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesion. *J Am Soc Nephrol* 10: 822–832, 1999.
139. Takahashi M, Suzuki M, Kushida K, Miyamoto S, and Inoue T. Relation between pentosidine concentrations in serum and urine and activity of rheumatoid arthritis. *Br J Rheumatol* 36: 637–642, 1997.
140. Tanaka N, Yonekura H, Yamagishi S, Fujimori S, Fujimori H, Yamamoto Y, and Yamamoto H. The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor α through nuclear factor- κ B, and by 17 β -estradiol through Sp1 in human vascular endothelial cells. *J Biol Chem* 275: 25781–25790, 2000.
141. Tanji N, Markowitch G, Fu C, Kislinger T, Taguchi A, Pischetsrieder M, Stern D, Schmidt AM, and d'Agati V. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *J Am Soc Nephrol* 11: 1656–1666, 2000.
142. Thallas-Bonke V, Lindschau C, Rizkalla B, Bach LA, Boner G, Meier M, Haller H, Cooper ME, and Forbes JM. Attenuation of extracellular matrix accumulation in diabetic nephropathy by the advanced glycation end product cross-link breaker ALT-711 via a protein kinase C- α -dependent pathway. *Diabetes* 53: 2921–2930, 2004.
143. Thornalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* 419: 31–40, 2003.
144. Twigg SM, Cao Z, McLennan SV, Burns WC, Brammar G, Forbes JM, and Cooper ME. Renal connective tissue growth factor induction in experimental diabetes is prevented by aminoguanidine. *Endocrinology* 143: 4907–4915, 2002.
145. Ueda Y, Miyata T, Hashimoto T, Yamada H, Izuhara Y, Sakai H, and Kurokawa K. Implication of altered redox regulation by antioxidant enzymes in the increased plasma pentosidine, an advanced glycation end product, in uremia. *Biochem Biophys Res Commun* 245: 785–790, 1998.
146. Van Boekel MA, Van den Bergh PJ, and Hoenders HJ. Glycation of human serum albumin: inhibition by Diclofenac. *Biochim Biophys Acta* 1120: 201–204, 1992.
147. Vlassara H, Li YM, Imani F, Wojciechowitz D, Yang Z, Liu FT, and Cerami A. Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE). A new member of the AGE-receptor complex. *Mol Med* 1: 634–646, 1995.
148. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, and Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. *Proc Natl Acad Sci USA* 91: 11704–11708, 1994.
149. Voziyan PA, Khalifah RG, Thibaudeau C, Yildiz A, Jacob J, Serianni AS, and Hudson BG. Modification of proteins in vitro by physiological levels of glucose. Pyridoxamine inhibits conversion of Amadori intermediate to advanced glycation end-products through binding of redox metal ions. *J Biol Chem* 278: 46616–46624, 2003.
150. Wautier MP, Chappey O, Corda S, Stern D, Schmidt AM, and Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 280: E685–E694, 2001.
151. Wautier JL and Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95: 233–238, 2004.
152. Wendt T, Tanji N, Guo J, Hudson BI, Bierhaus A, Ramasamy R, Arnold B, Nawroth PP, Yan SF, d'Agati V, and Schmidt AM. Glucose, glycation and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. *J Am Soc Nephrol* 14: 1383–1395, 2003.
153. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, Markowitz GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'Agati VD, and Schmidt AM. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am J Pathol* 162: 1123–1137, 2003.
154. Williams ME. Clinical studies of advanced glycation end product inhibitors and diabetic kidney disease. *Curr Diabetes Rep* 4: 441–446, 2004.
155. Williams ME, Bolton WK, Degenhardt TP, and Schotzinger R. A phase 2 clinical trial of pyridoxamine (pyridorin) in type 1 diabetic patients with overt nephropathy (PYR-206) (Abstract). *J Am Soc Nephrol* 14: 7A, 2003.
156. Wolf G. Cell cycle regulation in diabetic nephropathy. *Kidney Int* 77: S59–S66, 2000.
157. Wolf G, Sharma K, Chen Y, Ericksen M, and Ziyadeh FN. High glucose induced proliferation in mesangial cells is reversed by autocrine TGF- β . *Kidney Int* 42: 647–656, 1992.
158. Woffenbittel BH, Boulanger CM, Crijns FR, Huijberts MS, Poitevin P, Swennen GN, Vasan S, Egan JJ, Ulrich P, Cerami A, and Levy BI. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci USA* 95: 4630–4634, 1998.
159. Xiang G, Schinzel R, Simm A, Münch A, Münch G, Sebekova K, Kasper M, Niwa T, Schmitz C, and Heidland A. Advanced glycation end products (AGEs)-induced expression of TGF- β 1 is suppressed by a protease in the tubule cell line LLC-PK1. *Nephrol Dial Transplant* 16: 1562–1569, 2001.
160. Xu D and Kyriakis JM. Phosphatidylinositol 3'-kinase-dependent activation of renal mesangial cell Ki-Ras and ERK by advanced glycation end products. *J Biol Chem* 278: 39349–39355, 2003.
161. Yamagishi S, Inagaki Y, Okamoto T, Amano S, Koga K, and Takeuchi M. Advanced glycation end products inhibit de novo protein synthesis and induce TGF- β overexpression in proximal tubular cells. *Kidney Int* 63: 464–473, 2003.
162. Yamagishi S, Inagaki Y, Okamoto T, Amano S, Koga K, Takeuchi M, and Makita Z. Advanced glycation end product-induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. *J Biol Chem* 277: 20309–20315, 2002.
163. Yan SD, Schmidt AM, Anderson M, Zhang A, Brett J, Zou Y, Pinsky D, and Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269: 9889–9897, 1994.
164. Yan SD, Zhu H, Zhu A, Golabek A, Du H, Roher A, Yu J, Soto C, Schmidt AM, Stern D, and Kindy M. Receptor dependent cell stress and amyloid accumulation in systemic amyloidosis. *Nat Med* 6: 643–651, 2000.
165. Yang CW, Vlassara H, Peten EP, He CJ, Striker GE, and Striker LJ. Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. *Proc Natl Acad Sci USA* 91: 9436–9440, 1994.

166. **Yeh CH, Sturgis L, Haidacher J, Zhang XN, Sherwood SJ, Bjercke RJ, Juhasz O, Crow MT, and Tilton RG.** Requirement for p38 and p44/42 mitogen activated protein kinases in RAGE-mediated nuclear factor- κ B transcription activation and cytokine secretion. *Diabetes* 50: 1495–1504, 2001.
167. **Yonekura H, Yamamoto Y, Sakurai S, Petrova R, Abedin M, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, and Yamamoto H.** Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 370: 1097–1109, 2003.
168. **Youssef S, Nguyen DT, Soulis T, Pangiotopoulos S, Jerums G, and Cooper M.** Effect of diabetes and aminoguanidine therapy on renal advanced glycation end-product binding. *Kidney Int* 55: 907–916, 1999.
169. **Zheng F, Cai W, Mitsuhashi T, and Vlassara H.** Lysozyme enhances renal excretion of advanced glycation end products in vivo and suppresses adverse AGE-mediated cellular effects in vitro: a potential AGE sequestration therapy for diabetic nephropathy. *Mol Med* 7: 737–747, 2001.
170. **Zhou G, Cai L, and Cai L.** Advanced glycation end-products induce connective tissue growth factor-mediated fibrosis predominantly through transforming growth factor β -independent pathway. *Am J Pathol* 165: 2033–2043, 2004.
171. **Zhou Z, Wang K, Penn MS, Marso SP, Lauer MA, Forudi F, Zhou X, Qu W, Lu Y, Stern DM, Schmidt AM, Lincoff AM, and Topol EJ.** Receptor for AGE (RAGE) mediates neointimal formation in response to arterial injury. *Circulation* 107: 2238–2243, 2003.
172. **Ziyadeh FN.** Mediators of diabetic renal disease: the case for TGF- β as the major mediator. *J Am Soc Nephrol* 15: S55–S57, 2004.
173. **Ziyadeh FN, Sharma K, Ericksen M, and Wolf G.** Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor- β . *J Clin Invest* 92: 536–542, 1994.

