Uremic Toxicity of Advanced Glycation End Products in CKD

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ABSTRACT

Advanced glycation end products (AGEs), a heterogeneous group of compounds formed by nonenzymatic glycation reactions between reducing sugars and amino acids, lipids, or DNA, are formed not only in the presence of hyperglycemia, but also in diseases associated with high levels of oxidative stress, such as CKD. In chronic renal failure, higher circulating AGE levels result from increased formation and decreased renal clearance. Interactions between AGEs and their receptors, including advanced glycation end product–specific receptor (RAGE), trigger various intracellular events, such as oxidative stress and inflammation, leading to cardiovascular complications. Although patients with CKD have a higher burden of cardiovascular disease, the relationship between AGEs and cardiovascular disease in patients with CKD is not fully characterized. In this paper, we review the various deleterious effects of AGEs in CKD that lead to cardiovascular complications and the role of these AGEs in diabetic nephropathy. We also discuss potential pharmacologic approaches to circumvent these deleterious effects by reducing exogenous and endogenous sources of AGEs, increasing the breakdown of existing AGEs, or inhibiting AGE-induced inflammation. Finally, we speculate on preventive and therapeutic strategies that focus on the AGE-RAGE axis to prevent vascular complications in patients with CKD.


Advanced glycation end products (AGEs) constitute a heterogeneous group of compounds derived from the nonenzymatic glycation of proteins, lipids, and nucleic acids through a complex sequence of reactions referred to as the Maillard reaction.1,2

Generation of AGEs

Protein glycation is initiated by a nucleophilic addition reaction between a free amino group from a protein and a carbonyl group from a reducing sugar, with the formation of an unstable, freely reversible Schiff base. This base can be rearranged to form a more stable intermediate called an Amadori product, which in the presence of a transition metal, is oxidized to yield the final AGE (Figure 1) (reviewed in ref. 3). AGEs can also be formed by autoxidation of glucose and oxidative stress. Humans are exposed to exogenous sources of AGE (diet4 and cigarette smoke5) and endogenous sources of AGE when the organism is exposed to high levels of glucose, such as in diabetes.6 AGE precursors include the 1,2-dicarbonyl compounds glyoxal and methylglyoxal (MG), a highly reactive dicarbonyl compound.7 At least 20 different types of AGE have been described: N-carboxymethyllysine (CML), pentosidine, and hydroimidazolone are among the best characterized, are relatively nonreactive, and serve as markers of AGE accumulation in several tissues.8,9 AGEs can be degraded by enzymes, such as glyoxalase I (Glo-1) and II (Glo-2).10 Glo-1 detoxifies reactive α-oxoaldehyde, removing deleterious species, such as MG.11 AGEs can also be modified by innate defense machineries, such as lysozyme, which sequesters AGEs and accelerates their renal excretion in vivo,12 and receptor-dependent uptake and degradation.13

Receptors for AGEs

Advanced glycation end products receptor 1 (AGER1) binds AGEs14 and leads to their sequestration and detoxification, thus

Published online ahead of print. Publication date available at www.jasn.org.

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Reducing AGE levels in the intracellular and extracellular spaces, resulting in antioxidant properties. Advanced glycation end product–specific receptor (RAGE), another well characterized receptor for AGEs, is a multiligand transmembrane cell surface receptor that belongs to the Ig protein superfamily and binds many ligands, including AGEs. In the absence of disease, RAGE is usually expressed at very low levels in various cell types. In several diseases, such as diabetes and autoimmune/inflammatory diseases, RAGE expression is elevated, whereas AGER1 levels are decreased, resulting in suppression of the antioxidant defense system and increased levels of pro-oxidant mechanisms. The soluble truncated form of RAGE lacks the full-length transmembrane domain of the receptor. Soluble advanced glycation end product–specific receptor (sRAGE) can be produced by alternative splicing (endogenous secretory advanced glycation end product–specific receptor [esRAGE]) or proteolytic cleavage mediated by metalloproteinase (sRAGE). It is consequently released into the extracellular space, where it can sequester AGEs. High sRAGE levels are associated with an increased incidence of CKD before but not after adjustment for baseline kidney function, suggesting that either circulating sRAGE levels are directly affected by an impaired kidney filtration or inversely, circulating sRAGE directly affects kidney function. Additional studies are needed to elucidate the mechanisms of the association between sRAGE and kidney disease.

**PATHOPHYSIOLOGIC EFFECTS OF AGES**

**Mechanisms**

Accumulation of AGEs in patients with CKD has been shown to result from inflammation, oxidative stress, and diet. AGEs are proinflammatory and pro-oxidative compounds that play a role in the high prevalence of endothelial dysfunction and subsequent cardiovascular disease (CVD) in patients with CKD. Oxidative stress induced by reactive oxygen species (ROS) is associated with atherosclerosis and cardiovascular morbidity in patients with CKD. AGEs increase the levels of ROS through activation of NADPH oxidase and mitochondrial pathways in both a receptor-dependent manner (i.e., through RAGE) and a receptor-independent manner. In patients with type 2 diabetes mellitus, circulating AGE levels are correlated with RAGE mRNA expression and oxidative markers, such as protein carbonyl formation, advanced oxidation protein product generation, and lipid peroxidation. Reciprocally, high levels of ROS lead to increased levels of AGEs, because another cause of AGE formation in uremia is the increased oxidative stress generated by an imbalance between oxidized glutathione and GSH levels as well as changes in antioxidant systems, such as superoxide dismutase (SOD)/peroxidase. In fact, oxidative stress is closely linked to glycation, because GSH depletion also decreases the in situ activity of Glo–1, thereby increasing glyoxal and MG concentrations. Interestingly, AGER1 is downregulated by elevated AGE levels. Furthermore, AGEs have been shown to increase the oxidation of LDLs—a key stage in the development of atherosclerosis.
are, therefore, more susceptible to oxidation, are less effectively cleared from the circulation, and also, promote the formation of antibodies that bind AGEs localized in the vessel wall, which amplifies the development of vascular inflammation and atherosclerosis. Overexpression of Glo-1 in an animal model has been shown to have beneficial vascular effects, decrease ROS, and protect against formation of atherogenic LDL.

Numerous studies have shown that the nuclear factor erythroid 2–related factor (Nrf2) plays an important role in the antioxidant response. Under basal conditions, Nrf2 is associated in the cytoplasm with the repressor kelch like ECH–associated protein1 (Keap1), which promotes its ubiquitination for degradation. However, in response to oxidative stress, Nrf2 is released from Keap1 and translocates to the nucleus to induce the expression of genes encoding antioxidant and detoxifying molecules by binding to the antioxidant response element region of their promoter. Moreover, it has been shown that targeting Nrf2 ameliorates oxidative stress. Several studies have shown that some Nrf2 target antioxidant genes (SOD and glutathione peroxidase) are decreased in animal models of CKD and patients with CKD, which may consequently aggravate oxidative stress and inflammation. MG induces oxidative stress partly by negatively affecting Nrf2. Interestingly, one of the targets of the Nrf2/Keap system is Glo-1. In Nrf2−/− mice, Glo-1 mRNA is decreased, whereas MG urine excretion is increased. Transcriptional control of Glo-1 by Nrf2, therefore, provides protection against dicarbonyl glycation.

Inflammation

AGEs have been shown to amplify inflammatory responses in patients with CKD through RAGE, because the RAGE-AGE interaction activates the redox–sensitive transcription factor NF-κB, which leads to gene expression and the release of proinflammatory molecules, such as IL-1α, IL-6, and TNF-α. The in vitro use of human recombinant lysozyme significantly reduced AGE–induced IL-6 mRNA. It has recently been shown that targeting Nrf2 ameliorates inflammation by inhibiting the NF-κB pathway and probably, increasing Glo-1, which detoxifies reactive oxoaldehydes, such as MG. RAGE expression by activated endothelium also promotes leukocyte recruitment. Moreover, AGEs delay spontaneous apoptosis of monocytes and consequently, contribute to the progression of CKD.

Figure 2. Pathophysiologic effects of AGEs. cGMP, cyclic guanosine monophosphate; ECM, extracellular matrix; eNOS, endothelial NO synthase; EPC, endothelial progenitor cell; O2°, superoxide anion; ONOO°, peroxynitrite.
development of inflammatory responses.\textsuperscript{61} A recent prospective study of patients with AKI and sepsis showed that the association of RAGE and inflammatory mediators contributes to endothelial dysfunction.\textsuperscript{62} Moreover, exposure of the endothelium to uremic plasma results in a time- and CKD stage–dependent increase in expression of monocyte chemoattractant protein-1 and IL-8, suggesting the presence of a link between systemic inflammation and uremic toxicity.\textsuperscript{63} In addition to its antioxidant properties, AGER1, by increasing the degradation of AGEs, is a negative regulator of the inflammatory response in inflammatory and parenchymal cells, such as vascular smooth muscle and glomerular mesangial cells.\textsuperscript{64}

Cardiovascular Effects of AGEs

Patients with CKD have a higher CVD burden than the general population, and renal failure is associated with elevated levels of circulating AGEs.\textsuperscript{65,66} AGEs have an important cardiovascular effect, because AGE accumulation leads to endothelial dysfunction, arterial stiffness, myocardial changes, immune system dysregulation, and atherosclerosis progression. Studies of the relationship between AGEs and CVD in ESRD have yielded conflicting results.\textsuperscript{67–75} It is noteworthy that many different AGEs have been identified with widely varying tissue localization and composition, which could, therefore, explain these conflicting results. However, recent studies have shown a strong link between serum sRAGE levels and cardiovascular risk factors and disease.\textsuperscript{67,68} The main deleterious effects of AGEs in the cardiovascular system are described below.

Endothelial Dysfunction

Endothelial dysfunction, an early marker of atherosclerosis,\textsuperscript{77} is predictive of the occurrence of cardiovascular events. In CKD, endothelial dysfunction begins early in the course of the disease independently of traditional cardiovascular risk factors.\textsuperscript{78}

Plasma levels of endothelial cell activation markers are elevated in advanced CKD, and exposure of the endothelium to uremic plasma increases soluble vascular cell adhesion molecule-1 (VCAM-1) expression.\textsuperscript{63} AGE-RAGE–induced oxidative stress is central to VCAM-1 induction, because anti-RAGE antibodies, sRAGE, and N-acetylcysteine inhibit VCAM-1 expression.\textsuperscript{79} It has recently been shown that chronic CML ingestion induced RAGE–dependent endothelial dysfunction in mice.\textsuperscript{80} Furthermore, Glo-1 overexpression decreases endothelial dysfunction in a diabetic rat model.\textsuperscript{81}

In patients with CKD, endothelial dysfunction results from increased endothelial injury and decreased endothelial repair\textsuperscript{82} caused by a decreased endothelial progenitor cell (EPC) count.\textsuperscript{83–89} Furthermore, circulating EPC levels in patients with ESRD are negatively correlated with skin autofluorescence (a marker of tissue-bound AGEs) but not with serum pentosidine levels.\textsuperscript{89,90} Recent studies have shown that AGEs impair EPC survival, differentiation, migration, and function.\textsuperscript{91–93}

One of the endothelium’s most important functions is the synthesis and release of endothelium–derived relaxing factors, such as nitric oxide (NO) and prostaglandin I2 (PGI2). Patients with CKD display impaired endothelium–dependent vasodilation.\textsuperscript{94} AGEs have been shown to impair NO production partly through increased mRNA degradation.\textsuperscript{96} In vitro studies show that sera from patients with CKD suppress endothelial NO synthase activity in cultured vascular endothelial cells.\textsuperscript{97,98} This inhibition of endothelial NO synthase activity is mediated by RAGE activation of peripheral mononuclear cells in patients with CKD.\textsuperscript{31} AGEs also inhibit NO by increasing NADPH oxidase expression, establishing a link between RAGE and chronic endothelial dysfunction.\textsuperscript{34,39,95} PGI2 has also been shown to be decreased by AGEs, such as glycated albumin in cultured microvascular endothelial cells.\textsuperscript{99}

Endothelial dysfunction is defined as an imbalance between vasodilating and vasoconstricting substances produced by or acting on the endothelium. The intracellular signaling molecule endothelin-1 (ET-1), best known as a potent endogenous vasoconstrictor peptide produced by endothelial cells, has an important role in the vasculature and is involved in the development of atherosclerosis.\textsuperscript{100} Serum levels of ET-1 are markedly elevated in patients with chronic renal failure,\textsuperscript{101,102} and ET-1 is involved in both the development and progression of CKD.\textsuperscript{103} Odetti et al.\textsuperscript{101} observed a significant positive correlation between pentosidine and ET-1 plasma levels in subjects undergoing chronic hemodialysis (HD). Recently, it has been shown that ET-1 transcription in cultured bovine aortic endothelial cells is controlled by the AGE–inducible, redox–sensitive transcription factor NF-κB.\textsuperscript{104} AGEs, therefore, play an important role in endothelial dysfunction by (1) decreasing levels of two important endothelium–dependent relaxing factors (NO and PGI2) and (2) increasing endothelial production of the potent vasoconstrictor ET-1.\textsuperscript{104}

Another important role of the endothelium is its function as a selective barrier between the blood and the surrounding tissues. AGEs have been shown to be associated with increased permeability of the endothelial layer.\textsuperscript{105,106}

Arterial Stiffness

Arterial stiffening occurs because of loss of compliance of the vessel wall\textsuperscript{107,108} and is an important independent risk factor for cardiovascular mortality in patients with CKD.

CKD not only accelerates the development of atherosclerosis but also, leads to excessive vascular calcification resulting from the inflammation and oxidative stress present in patients with CKD.\textsuperscript{109–113} Vascular calcification is an independent predictor of cardiovascular mortality,\textsuperscript{114,115} and one of its consequences is altered aortic compliance.\textsuperscript{116} AGE levels\textsuperscript{117} as well as tissue AGEs\textsuperscript{118} are associated with vascular calcification in patients with CKD, suggesting that increased AGE may contribute to vascular calcification. It has recently been shown, in an animal model, that AGE-induced calcification is mediated by RAGE and oxidative stress.\textsuperscript{119} Moreover, lower sRAGE levels were recently associated with carotid plaque calcification.\textsuperscript{76}

Arterial stiffness has a major role not only in the increase in systolic BP and pulse pressure, but also in the decrease
in diastolic BP. This BP alteration leads to left ventricular hypertrophy and abnormal pulse wave velocity (PWV), both of which are apparent at the early stages of CKD. Measurement of PWV, a noninvasive method for assessing arterial wall stiffness related to vascular calcification, is a strong independent predictor of cardiovascular mortality in patients with ESRD. Several studies suggest that AGEs are involved in arterial stiffness. For example, chronic CML ingestion induced RAGE-dependent arterial stiffness in mice. Zhou et al. showed a positive correlation between arterial stiffness and serum pentosidine levels. Moreover, skin autofluorescence (a marker of AGE accumulation) is associated with arterial PWV (and therefore, arterial stiffness) in patients with ESRD. This may be caused by the formation of irreversible cross-links between the end products and long-lived structural proteins, such as collagen and elastin.

**Immune System Dysregulation**

The immune system plays an important role in the development and progression of CVD. It has been shown that dysregulation of the immune system contributes to atherosclerosis in patients on HD. Several studies have suggested that AGEs can amplify local immune reactions in the kidney. Other studies in humans have shown that AGE formation and accumulation are partially responsible for the observed immune system dysregulation. Friedlander et al. showed a positive association between serum pentosidine and monocyte activation, which may contribute to elevated complication rates. It has also been shown that decreased AGE intake protects against loss of innate immunity.

**Diabetic Nephropathy**

Diabetic nephropathy (DN) is the leading cause of ESRD and the most common indication for RRT. The kidney has an important role in the metabolism of AGEs, because renal proximal tubule cells absorb AGEs from the glomerular filtrate and catabolize them. GFR is negatively correlated with not only serum AGE levels but also, RAGE mRNA expression in PBMCs. Lysozyme that sequesters AGEs reduces the severity of the early manifestations of DN, suggesting that AGEs play an important role in the pathogenesis of DN. Immunohistochemical studies in patients with DN have shown that AGEs accumulate in the mesangium and glomerular capillary wall. Horie et al. showed that patients with advanced DN exhibit enhanced CML accumulation in the expanded mesangial matrix and a thicker glomerular capillary wall in early nodular lesions and arterial walls (relative to the healthy kidney). Similar results were found with the AGE midazolam.

**Inhibition of Gastrointestinal Absorption**

In view of the many harmful effects of AGEs on cell function, it is essential to develop strategies designed to counteract their effects. Several pharmacologic treatment strategies targeting the AGE-RAGE system have been studied and in vivo for their potential to prevent AGE formation or local AGE accumulation. These therapeutic compounds can be divided into several classes as a function of their mechanism of action (Figure 3, Table 1): AGE absorption inhibitors, AGE formation inhibitors, AGE cross-link breakers, RAGE antagonists, and AGE binders, such as sRAGE. Although many compounds are currently being studied, only a few have entered clinical trials, and none have yet been approved for clinical use in patients with CKD.

**Decreasing Exogenous Sources of AGEs**

**Decreased Dietary AGE Intake**

Dietary AGE content is an important contributor to serum AGE accumulation in patients with CKD. As mentioned above, AGEs are generated during the thermal processing and storage of foods. A recent clinical study in patients with CKD showed that reducing dietary AGEs may lower oxidative stress and inflammation, restore AGER1 levels, and protect against innate immune dysfunction. Dietary restriction is, therefore, an effective, feasible, and economic method to reduce the levels of toxic AGEs and possibly, the associated cardiovascular mortality.

**Inhibition of Gastrointestinal Absorption**

Another option to reduce the intake of exogenous AGEs is to block or inhibit the gastrointestinal absorption of dietary AGEs. Several compounds have been widely studied, including the spherical carbon adsorbent AST-120 and sevelamer carbonate.

Administration of the oral adsorbent AST-120 before initiating dialysis has been shown to not only improve the survival rate in patients on HD but also, delay the onset of HD in patients with CKD. AST-120 is also associated with a reduction in carotid intima media thickness and arterial stiffness in patients with CKD and without diabetes. However, recent randomized placebo-controlled Evaluating Prevention of Progression In CKD (EPPIC) Trials of AST-120 in CKD did not support the benefit of adding AST-120 to standard therapy. AST-120 has been shown to bind AGE and effectively decrease plasma AGE levels. In a recent animal study, AST-120 was shown to reduce the indoxyl sulfate–induced decrease of Nrf2, which may, therefore, decrease formation of AGEs, such as MG, by increasing Glo-1.

Sevelamer carbonate is a nonabsorbable, noncalcium–based compound frequently used to lower blood phosphorus levels in patients with advanced CKD or ESRD. It has recently been shown that sevelamer can act as a pleiotropic drug by sequestering cystotoxic AGEs in the gut and thus, preventing their uptake. Moreover, sevelamer decreases CML levels, oxidative stress, and inflammation and increases AGER1 expression. The results of a randomized trial in
patients on HD showed that sevelamer slows calcification and suppresses pentosidine accumulation. Although long-term clinical studies in patients with CKD have not yet been performed, a short-term comparison of sevelamer with calcium carbonate showed that it lowered serum AGEs in patients on HD. Sirtuin1, an NAD+-dependent histone deacetylase that has anti-inflammatory, antiapoptotic, and antioxidant properties and prevents calcification and endothelial senescence, is decreased by AGEs, such as MG. In a recent clinical study in patients with type 2 diabetes mellitus and stages 2–4 diabetic kidney disease, sevelamer significantly increased antioxidant molecules, such as AGER1, Nrf2, and Sirtuin1, while decreasing pro-oxidant molecules, such as RAGE and AGEs. Lin et al. showed that online hemodiafiltration, a combination of both HF and HD, for >6 months in patients with uremia leads to a significant reduction in serum AGE levels. A recent study showed that convective therapies not only effectively cleared uremic toxins but also, decreased levels of inflammatory markers, such as IL-6.

PD, an important alternative to HD, also reduces AGEs formation. Advantageously, serum concentrations of AGEs, such as pentosidine, are lower in patients on PD than in patients on HD. However, despite higher levels of AGE excretion in patients on PD, free AGE levels in the PD effluent were elevated because of increased synthesis of free AGEs. Conventional PD fluid contains supraphysiologic concentrations of glucose and glucose degradation products.
Table 1. Clinical studies to decrease levels of AGEs in patients with CKD

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products (GDPs; generated during sterilization by heating of dialysis fluid) that can increase the formation of AGEs.\textsuperscript{172–174}

In patients on PD, high levels of AGE deposition in tissue correlate with glucose exposure.\textsuperscript{175} Nonglucose PD solutions or solutions containing low-GDP levels, such as icodextrin, may, therefore, minimize AGE formation.\textsuperscript{176–179}

The acidic pH and the hyperosmolality of conventional PD fluid can also damage the peritoneum and consequently, impair its function as a dialyzing membrane. Other alternatives (such as neutral pH and low–GDP peritoneal solutions\textsuperscript{180}) can also be used to decrease AGE formation during conventional PD.\textsuperscript{181}

Continuous flow PD is another option that also effectively reduces AGE formation.\textsuperscript{170}

Kidney Transplantation

Because AGEs are mainly excreted by the kidneys, serum AGE levels increase with declining kidney function.\textsuperscript{182} Kidney transplantation is, therefore, an efficient way of decreasing levels of uremic toxins, including AGEs. Thus, pentosidine levels started to fall within 4 weeks of kidney transplantation and reached normal values after 6 months.\textsuperscript{183} Studies showed that kidney transplantation decreases AGE accumulation in tissues,\textsuperscript{184,185} which was assessed by skin autofluorescence, thereby significantly reducing cardiovascular events in recipients of transplants compared with patients on dialysis.\textsuperscript{185}

Antioxidants

To decrease endogenous sources of AGEs, several compounds have been developed to attenuate glyoxidation and/or oxidative stress by sequestration of metal ions, reactive dicarbonyl compounds, ROS, and reactive nitrogen species. Thus, the use of antioxidants, such as vitamin E, vitamin A, and lipoic acid, has been studied \textit{in vitro} to counteract AGE effects.\textsuperscript{186}

**Reactive Dicarbonyl Scavengers**

Several compounds can trap reactive carbonyl intermediates (AGE precursors) and quench ROS.\textsuperscript{187,188} For example, the strongly nucleophilic Pimagedine (2-amino-guanidine) can scavenge reactive carbonyl intermediates in the Maillard reaction\textsuperscript{189,190} and also, decrease VCAM-1 expression in endothelial cells.\textsuperscript{191} Administration of aminoguanidine to patients with ESRD also led to a reduction of circulating LDL levels.\textsuperscript{39}

The vitamin B6 derivative pyridoxamine (registered as Pyridorin) is a post-Amadori inhibitor\textsuperscript{192} that traps reactive carbonyl and dicarbonyl compounds derived from Amadori compounds, thereby reducing AGE accumulation.\textsuperscript{193,194} Pyridoxamine also scavenges ROS and chelates metal ions (oxidation catalysts). In diabetic rats, pyridoxamine has been shown to decrease AGE and calcification.\textsuperscript{195} Polizzi \textit{et al.}\textsuperscript{196} showed that a combination of vitamins B1 and B6 decreases DNA glycation in patients with DN. However, an 8-week randomized, placebo–controlled study in 50 patients on HD found that the combination of pyridoxine and thiamine had no effect on plasma levels of AGEs and pentosidine.\textsuperscript{197}

**Aldose Reductase Inhibitors**

Aldose reductase is a multifunctional enzyme that reduces aldehydes, and it is involved in the polyol pathway of AGE formation. Inhibiting aldose reductase, therefore, decreases levels of AGE precursors and decreases AGE accumulation.\textsuperscript{305} Administration of the aldose reductase inhibitor epalrestat decreases serum AGE levels in patients with diabetes.\textsuperscript{206}

**Increasing the Breakdown of AGEs**

Agents that break AGE cross-links (reviewed in ref. 207) have been shown to improve arterial compliance in humans.\textsuperscript{208} Animal and human studies have shown that AGE cross–link breakers, such as ALT-711 and alagebrium, decrease AGE levels\textsuperscript{209,210} and tissue AGEs,\textsuperscript{211,212} reverse their effects,\textsuperscript{213–216} such as aortic stiffness, calcification,\textsuperscript{195} and extracellular matrix accumulation,\textsuperscript{212} and improve renal function by facilitating urinary excretion of the end products.
RAGE Inhibitors

Many different animal studies have shown that RAGE blockade by decreasing RAGE expression or RAGE competition reduces oxidative stress and endothelial dysfunction. Thus, sRAGE prevents the development of structural and functional characteristics of nephropathy in db/db mice. Recently, a receptor-based biosorbent designed to target AGE gave promising results to selectively deplete serum AGEs from human blood. This sRAGE–based extracorporeal therapy could be useful to selectively decrease serum AGEs and decrease inflammation in patients with diabetes and/or CKD.

Other Approaches

Other therapeutic approaches, such as calcium channel blockers, antidiabetic drugs, statins, and lysozyme, have also been used. Recent studies have also described the use of alternative medicines with antiglycation activities, such as extracts from certain plants like Moutan cortex and Azadirachta indica, which exert renoprotective effects by inhibition of the AGE-RAGE axis (Table 1). This section will focus on emerging therapeutic approaches, such as microRNAs (miRs) and Nrf2 and Glo-1 inducers.

MiRs

There is growing evidence that miRs play a key role in kidney physiology and contribute to both the induction and progression of CKD. miRs are ubiquitously expressed short noncoding RNAs comprising 20–22 nucleotides that regulate key biologic pathways and cellular functions by inhibiting gene expression through post-transcriptional repression of their target mRNAs. For example, miR21 has been shown to be reduced in AGE–induced endothelial cell apoptosis. AGES also strongly increased miR214 in monocytes from patients with chronic renal failure, which in turn, abolished AGE–induced cell survival, thereby limiting the inflammatory response. Identifying specific miRs that target the AGE-RAGE axis is, therefore, of interest to correct their expression by delivery of miR mimics to restore miR levels or inhibitors to block miR function. Chen et al. have recently shown that miR29b, which inhibits DN in db/db mice, was downregulated in response to AGEs and that its overexpression by gene therapy attenuated diabetic kidney disease. miRs have consequently emerged as a promising novel therapeutic strategy for the treatment of CKD. However, miR delivery and safety require additional investigation before clinical application.

Nrf2 and Glo-1 Inducers

Nrf2 signaling has been shown to have a protective role in renal injuries. Several Nrf2 target antioxidant genes, such as SOD, are decreased in CKD. Pharmacologic interventions activating Nrf2 could, therefore, be beneficial to protect the kidney in CKD, partly by ameliorating oxidative stress. Because another target of Nrf2 is Glo-1, targeting Nrf2 can also decrease AGEs, such as MG. Naturally occurring Nrf2 activators have been described in animal studies (reviewed in ref. 241). For example, ankaflavin, a natural peroxisome proliferator-activated receptor-γ agonist, has been shown to upregulate Nrf2 and protect against MG-induced diabetes in vivo. Monascin, obtained from Monascus-fermented products, has recently been shown to possess anti-inflammatory and antioxidant properties by decreasing RAGE and increasing heme oxygenase 1 by upregulation of Nrf2. Dimerumeric acid, another product extracted from Monascus, has an inhibitory effect on CML–induced RAGE signals by Nrf2-mediated attenuation of oxidative stress in hepatic cells. It also decreases hepatic Glo, thereby decreasing serum and hepatic AGEs in MG-treated mice. These widely used plant–derived Nrf2 activators have been shown to be safe with shown health benefits in human subjects, suggesting that they could be beneficial for the treatment of CKD. Bardoxolone methyl, a synthetic Nrf2 activator, has been tested as a therapeutic agent in clinical trials. Despite promising results in phase II trials, a recent study showed that it contributes to higher rates of cardiovascular events in susceptible patients with an increased risk of heart failure at baseline. Additional studies are, therefore, needed to evaluate the safety of these compounds. However, Nrf2 activators seem to be a promising new therapeutic strategy to improve or delay kidney dysfunction.

CONCLUSION

AGEs have been widely described in hyperglycemic conditions, such as diabetes; however, they can also be generated in conditions associated with elevated levels of oxidative stress, such as CKD. The kidney has an important role in AGE metabolism, because CKD is associated with AGE accumulation with declining kidney function. In this review, we describe the mechanisms of AGE formation and the various pathologic effects of AGEs. The experimental data available to date emphasize the harmful effects of AGEs on vascular function (with endothelial dysfunction, elevated oxidative stress, and arterial stiffness) and the immune system. We also describe the various potential therapeutic strategies designed to decrease exogenous and endogenous sources of AGE precursors or increase the breakdown of AGEs. A large body of evidence has shown that the interaction between AGEs and RAGE plays a key role in vascular damage. Inhibiting the AGE-RAGE axis by means of RAGE inhibitors could become a novel therapeutic strategy. Of interest are the recent new potential therapeutic strategies emerging from in vitro and in vivo studies, such as Nrf2 and Glo-1 inducers as well as miRs regulation.

We provide an overview of the pathobiology of AGEs and information on how AGE accumulation is involved in vascular damage in patients with CKD. Additional preclinical and clinical studies are,
nevertheless, needed to (1) determine the relevance of targeting AGEs to reduce the complications of CKD and (2) identify new therapeutic interventions that may reduce or delay morbidity and mortality in this population.

DISCLOSURES

H.V. and G.E.S. received support for an investigator-initiated trial of Sevelamer in the management of diabetic kidney disease from Sanofi.

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BRIEF REVIEW


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