

Review

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Advanced glycation end products mediated cellular and molecular events in the pathology of diabetic nephropathy

DOI 10.1515/bmc-2016-0021

Received August 9, 2016; accepted September 18, 2016

Abstract: Diabetic nephropathy (DN) is a major cause of morbidity and mortality in diabetic patients and a leading cause of end-stage renal disease (ESRD). Degenerative changes such as glomerular hypertrophy, hyperfiltration, widening of basement membranes, tubulointerstitial fibrosis, glomerulosclerosis and podocytopathy manifest in various degrees of proteinuria in DN. One of the key mechanisms implicated in the pathogenesis of DN is non-enzymatic glycation (NEG). NEG is the irreversible attachment of reducing sugars onto free amino groups of proteins by a series of events, which include the formation of Schiff's base and an Amadori product to yield advanced glycation end products (AGEs). AGE modification of client proteins from the extracellular matrix induces crosslinking, which is often associated with thickening of the basement membrane. AGEs activate several intracellular signaling cascades upon interaction with receptor for AGEs (RAGE), which manifest in aberrant cellular responses such as inflammation, apoptosis and autophagy, whereas other receptors such as AGE-R1, AGE-R3 and scavenger receptors also bind to AGEs and ensue endocytosis and degradation of AGEs. Elevated levels of both serum and tissue AGEs are associated with adverse renal outcome. Increased evidence supports that attenuation of AGE formation and/or inhibition of RAGE activation manifest(s) in improving renal function. This review provides insights of NEG, discusses the cellular and molecular events triggered by AGEs, which manifest in the pathogenesis of DN including renal fibrosis, podocyte

epithelial-mesenchymal transition and activation of renin-angiotensin system. Therapies designed to target AGEs, such as inhibitors of AGEs formation and crosslink breakers, are discussed.

Keywords: AGEs; anti-glycating agents; diabetic nephropathy; fibrosis; non-enzymatic glycation.

Introduction

Proteins and polypeptides are routinely subjected to several post-translational modifications during their lifetime, which includes phosphorylation, glycosylation, deamination, ubiquitination and sumoylation. These post-translational modifications may facilitate proteins to achieve proper folding and sorting into distinct cellular compartments, elicit intracellular signaling and confer stability during stress conditions and degradation and regulation of their intracellular levels. Proteins are also susceptible to post-translational modifications that could alter their structure, function, and half-life during normal aging and pathological conditions such as diabetes. One such post-translational modification is non-enzymatic glycation (NEG, also called non-enzymatic glycosylation). NEG of client proteins ensue crosslinking and aggregation that may adversely affect the structural and functional properties of proteins. In addition to affecting the physicochemical properties of the afflicted proteins, NEG also induces aberrant cellular signaling, activation of transcription factors and alter gene expression profiles. The final products that accumulate owing to NEG are termed Advanced Glycation End Products (AGEs). Although NEG occurs at a lower rate over a lifetime, it occurs more rapidly in clinical conditions such as diabetes and is implicated in the pathophysiology of several complications of diabetes and other age-related disorders such as cataract, renal failure, cardiovascular complications, and Alzheimer's disease. Tissue AGEs correlate very tightly with early kidney, eye, and nerve disease in patients with diabetes mellitus (DM). The focus of this review is

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to provide insights of chemical reactions of NEG and AGE formation, details about predominant client proteins affected by NEG, fate of AGEs, details about the interaction between AGEs and their receptors and down-stream signaling cascades. Further, we discuss the mechanism of AGE-mediated cellular and molecular events that promote pathological events during diabetic nephropathy (DN). Possible therapeutic and dietary interventions to prevent AGE formation and to cleave AGE crosslinks will also be discussed.

Chemical reactions of non-enzymatic glycation (NEG)

In 1912, the French chemist Louis-Camille Maillard reported for the first time the formation of brown colored substances during the enzyme-free reaction between reducing sugars and glycine (1). The complex inter-related reactions between the free amino group and reducing sugars are known as the Maillard reaction, which is largely considered as a browning reaction that takes place in the processing of foods and beverages (2). The significance of the Maillard reaction was dormant for a very long time till browning reactions were identified *in vivo* by Monnier and Cerami in 1981 (3). The formation of fluorescent yellow pigments and crosslinks upon incubation of eye lens proteins with reducing sugars was reported, similar to those reported in aging and cataractous lenses (3). The classical reaction of NEG occurs through the covalent bonding of reducing sugars (e.g. glucose or fructose) with free amino groups of proteins at N-terminal amino acid residues and/or ϵ -amino groups of lysine and arginine. NEG preferably occurs at amino groups that are either close ($\sim 5 \text{ \AA}$) to an imidazole residue or a part of lysine doublet (4). The initial reaction between the reactive carbonyl group and a free amino group of a protein results in the formation of a labile Schiff's base (Figure 1A). The initial Schiff's base (aldimine) undergoes spontaneous rearrangements to a relatively stable ketoamine (1-amino-1-deoxy-2-ketose), which is known as an Amadori product, after Mario Amadori, an Italian scientist, who attempted to characterize the products of a condensation reaction between glucose and *p*-phenetidine (5). Both the Schiff's base and Amadori product can undergo further reactions with accessible lysine/arginine residues of the client proteins and form AGEs (6, 7). The rate at which the Schiff's base forms takes hours to days, whereas it may take days to weeks and weeks to years to progress to Amadori products and the formation of AGEs,

respectively (8). The term AGEs was coined by Cerami to refer to fluorescent pigments arising from the degradation of fructosamine, which can crosslink proteins (9). The term AGEs is used to describe any protein-bound adduct detected after the formation of the initial Schiff's base/Amadori product, and that could be considered as the final product of Maillard reaction. AGEs may result from a one-step conversion from the Amadori product, as it happens in the case of carboxymethyllysine (CML), or a series of complex reactions leading to a crosslink such as pentosidine and glyoxal-lysine dimer (GOLD) (Figure 2A). In addition, the oxidative breakdown of Amadori products can lead to the formation of reactive carbonyls such as glyoxal, methyl-glyoxal, and 3-deoxy-glucosone (3DG). These carbonyl compounds can, in turn, react with free amino groups of proteins to form intermediate glycation products. A series of reactions including dehydration, successive β -eliminations, and condensation reactions of both Amadori products and intermediate glycation products eventually result in the formation of irreversible inter- or intra-protein crosslinking AGEs that could persist for the lifetime of the modified substrate. John Hodge summarized the complex network of NEG reactions that take place during food processing and storage in his seminal review (10). The formation of AGEs from autoxidation of Amadori products is known as 'Hodge pathway' and cleavage of dicarbonyl compounds from Schiff's base refers to 'Namiki pathway' (Figure 1B). Alternatively, the formation of dicarbonyls from autoxidation of glucose, ribose, fructose, and glyceraldehyde is known as 'Wolff pathway' (11).

Advanced glycation end products

The AGEs comprise a large number of heterogeneous chemical structures and a majority of them have a crosslinking propensity between proteins, which alter their structure and function. AGEs are relatively insoluble, yellow-brown in color and a majority of them exhibit a fluorescence phenomenon. CML, pentosidine, argpyrimidine, pyrrolidine, imidazolone, 2-(2-furoyl)-4(5)-furyl-1H-imidazole (FFI), glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD), 3-deoxyglucosone-lysine dimer (DOLD), carboxyethyl-lysine, fructose-lysine, methylglyoxal-derived hydroimidazolones are well-known AGEs (Table 1). Both biochemical and immunohistochemical studies suggested that CML modifications of proteins are predominant AGEs that accumulate in the majority of tissues from diabetic subjects (12, 13). CML is

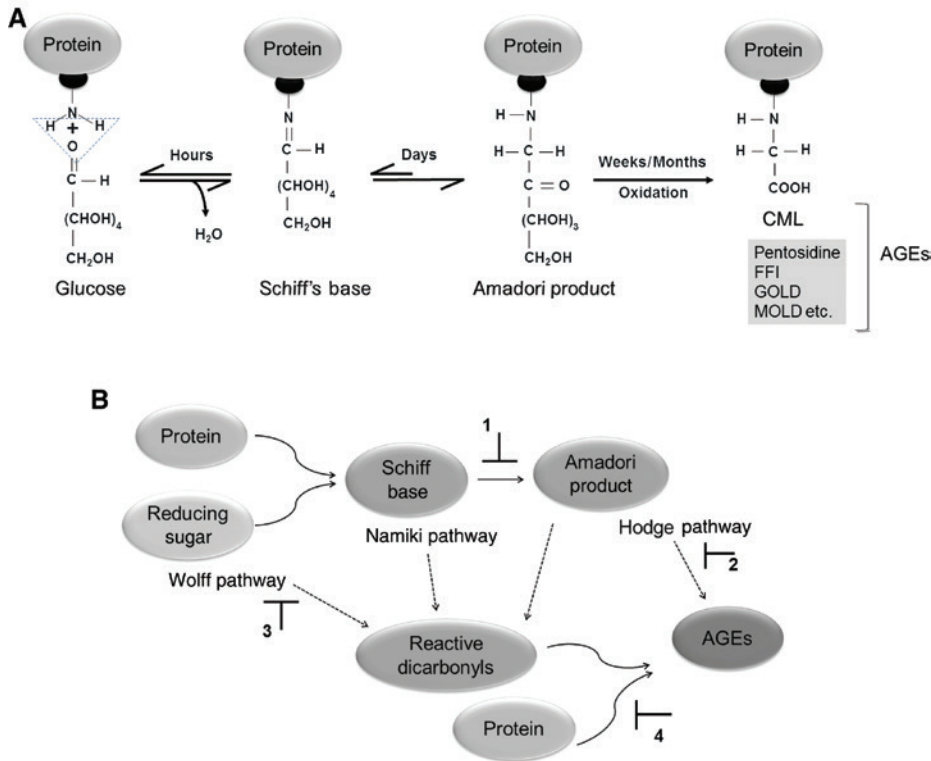


Figure 1: Mechanism and pathways of formation of advanced glycation end products.

(A) Classical reactions in the formation of advanced glycation endproducts (AGEs). The initial nonenzymatic interaction between the highly reactive carbonyl group of glucose with a free amino group on proteins creates a reversible Schiff's base, which spontaneously undergoes rearrangement itself into a partially reversible Amadori product. Amadori products rearrange to form heterogeneous AGEs including CML, pentosidine, FFI, MOLD and GOLD. (B) A complex network of NEG reactions. Highly reactive carbonyl intermediates (glyoxal, methylglyoxal and 3-deoxyglucosone) can be formed from the auto-oxidation of monosaccharides (e.g. glucose: Wolff pathway) or from Schiff's base (Namiki pathway) or from Amadori products (Hodge pathway). The highly reactive intermediates that are formed by these three pathways can react with free amino groups to form diverse AGEs. The possible sites that could be targeted to inhibit NEG and to prevent AGEs mediated complications are indicated in numbers [1–4].

nonfluorescent protein adduct and it can be formed by the oxidative degradation of Amadori products or the direct reaction of glyoxal with lysine. Quantification of CML levels is considered as an index of AGE content in food, and CML is the major epitope of the commonly used polyclonal antibodies employed to detect AGEs. On the other hand, pentosidine is a fluorescent glycoxidation product that forms protein-protein crosslinks. It is composed of an arginine and a lysine residue crosslinked to a pentose (14, 15). Carbonyl compounds (glyoxal, methylglyoxal, and 3-DG) derive from both oxidative degradation or auto-oxidation of Amadori products and are highly reactive molecules leading to protein crosslinks (16). AGEs such as carboxymethyl-hydroxy-lysine, carboxyethyl-lysine, fructose-lysine, methylglyoxal-derived hydroimidazolones, and pyrraline form non-fluorescent protein adducts, while GOLD and MOLD form nonfluorescent protein crosslinks.

Among commonly available sugars, glucose exhibits the slowest glycation rate, while intracellular sugars such

as fructose and glucose-6-phosphate form AGEs at a much faster rate and form heterogeneous crosslinking products (17). Reactive intermediates produced from degraded AGEs can bind and form 'second'-generation AGEs. Intermediate AGEs such as glyoxals, glycolaldehydes, and hydroxyl aldehydes can be formed during oxidation of both carbohydrates and fatty acids. The increase in glycoxidation and lipoxidation in diabetes contribute to the accelerated accumulation of carbonyl compounds in addition to NEG, and such a condition is generally referred to as carbonyl stress. The intermediates arising as a result of carbonyl stress also react with free amino groups of proteins to form AGEs. Therefore, apart from the direct action of reducing sugars with free amino groups, AGEs can also be formed via distinct oxidative reactions. Formation of AGEs is not only restricted to proteins, but lipoproteins and nucleic acids are also susceptible. It is noteworthy that AGEs can also be exogenously ingested through food, particularly from baked and processed food (18, 19). AGEs in cooked

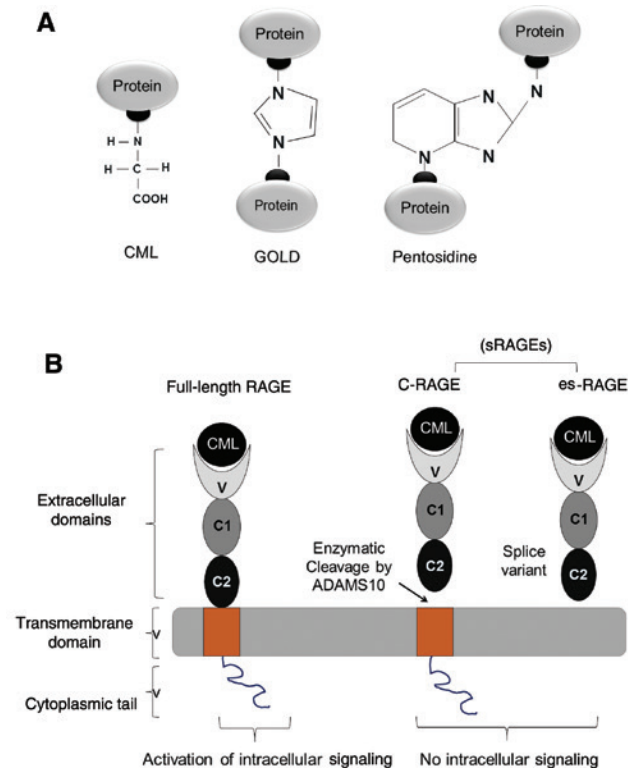


Figure 2: Schematic representation of predominant AGEs and receptors for AGEs (RAGE).

(A) AGEs can exist as protein adducts (CML) or AGEs can crosslink proteins (GOLD and pentosidine). (B) Structure of full-length RAGE and soluble RAGE (sRAGE). sRAGE derived via proteolytic cleavage of full-length RAGE by ADAMS 10, known as cleaved (cRAGE) or via alternative splicing of RAGE mRNA lacking the cytoplasmic tail and transmembrane domains known as endogenous secretory RAGE(esRAGE). Full-length RAGE, upon interaction with a ligand (for example, CML), elicit intracellular signaling, whereas sRAGE fails to transduce intracellular signaling and forms a natural defense mechanism to combat AGEs.

Table 1: Commonly found AGEs in biological systems and corresponding carbohydrate source and target amino acid.

Carbohydrate source	Target amino acid	AGEs
Glucose, Threose	Lysine	CML
Ribose	Lysine + Arginine	Pentosidine
Methylglyoxal	Arginine	Argpyrimidine
3-Deoxyglucosone	Arginine	Imidazolone
Methylglyoxal	Lysine	Carboxyethyl lysine
Glyoxal	Lysine	GOLD
Methylglyoxal	Lysine	MOLD
3-Deoxyglucosone	Lysine	DOLD

CML, carboxymethyllysine; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer; DOLD, 3-deoxyglucosone-lysine dimer.

food remain active in the circulation after oral uptake. Although AGEs accumulate endogenously at an accelerated rate during diabetes, AGEs are also formed at lower

rates during normal metabolic processes. However, they can sequester by the natural anti-AGE cellular defense system of the host. Environmental factors such as diet, smoking, and consumption of alcohol influence the rate of AGE accumulation.

Tissue targets of AGE modification

Free AGEs and protein-bound AGEs are found in several sites including plasma, vasculature, eye lens, retina and various renal compartments including mesangium, glomerulus, and basement membrane. Both extracellular and intracellular proteins are susceptible to modification with AGEs. In general, proteins and polypeptides have a limited life span and are periodically degraded and resynthesized. Exceptions to this are proteins that are stable throughout the lifetime of the individual, such as crystallins in the eye lens. Proteins with a relatively long biological half-life are expected to be more susceptible to modification with AGEs. Extracellular matrix (ECM) proteins such as collagen, laminin, elastin, and plasma proteins including hemoglobin and albumin, with a relatively short half-life, are also prone to NEG and crosslinking. Nevertheless, proteins in *E. coli* cultured under normal growth were shown to undergo NEG (20).

The accumulation of AGEs in eye lens crystallins contribute to the age-related incidence of brown color, fluorescence and decreased solubility of lens crystallins, thus contributing to senile cataract. α -crystallin, a predominant soluble eye lens protein, upon glycation with either reducing sugars such as glucose, fructose, glucose-6-phosphate or sugar derivatives such as methylglyoxal undergo crosslinking and form a range of high-molecular-weight aggregates (17, 21). Glycated α -crystallin displayed reduced chaperoning activity compared to unmodified α -crystallin to prevent thermal or stress-induced aggregation of co-lenticular proteins and substrate proteins. Reduced chaperoning activity of glycated α -crystallin is implicated in the development of diabetic cataract and impaired vision (21, 22).

Collagen and hemoglobin are extensively studied proteins for modification with glycation. Glycation results in decreased solubility and flexibility of collagen and is resistant to degradation by proteases. Glycation with glucose abolishes the positive charge of lysine and the formation of CML imparts a negative charge on lysine. Perturbations in charge profile of collagen monomers affect their aggregation into fibers and normal architecture of basement membrane (23, 24). In addition to affecting

inter-monomeric interactions, glycation of collagen alters its interaction with other components of the ECM such as proteoglycans, vitronectin, and laminin. In the case of glycated hemoglobin (HbA_{1c}), NEG occurs between glucose and the free amino group at N-terminal valine of the β -chain. HbA_{1c} is a measure to predict the extent and duration of glycemic control. Unlike ECM proteins, HbA_{1c} is more prone to proteolytic degradation compared with native hemoglobin. The life span of erythrocytes decreases by ~7 days for every 1% increase in HbA_{1c} levels. Free radicals arising as a consequence of HbA_{1c} degradation manifest in a plethora of events such as (a) increased fragility and decreased life span of erythrocytes, (b) erythrocyte aggregation, and (c) increased viscosity of blood and reduced blood flow.

Other than hemoglobin, plasma proteins including low-density lipoproteins (LDL) and immunoglobulins also undergo glycation. NEG alters LDL clearance and increases susceptibility to oxidative modifications. Macrophages have more affinity for uptake of glycated LDL over normal LDL via scavenger receptors and these events ultimately manifest in foam cell formation, which is a characteristic of atherosclerotic plaques (25). AGEs were also shown to alter the endothelial surface from an anti-coagulant nature to a pro-coagulant nature by reducing thrombomodulin expression and the concomitant induction of tissue factor expression (26). Besides these popular targets of NEG, several other proteins are also vulnerable to NEG and AGE accumulation. Glycated laminin has decreased affinity to polymerize and is unable to bind collagen and heparin sulfate proteoglycans, thus affecting the integrity of matrix interactions (27). NEG-mediated crosslinking of structural proteins and the resultant accumulation of AGEs contribute to the long-term complications of diabetes such as cataract, retinopathy, neuropathy, nephropathy, amyloidosis, and Alzheimer's disease. Histones, which play an important role in chromatin organization and epigenetic regulation of gene expression are vulnerable to NEG as evidenced by the accumulation of CML, pentosidine, imidazolone, and FFI (28). Among several histones, H1 and H2B are extensively subjected to glycation and all of the identified glycated residues were lysine (28). Glycation of histones results in structural alterations, which are speculated to alter the integrity of chromatin structure and function. Carboxyethyl guanine is reported to contribute to mutations and DNA transpositions (29). Glycosylation of immunoglobulins (IgG, IgA, and IgM) was noticed in both type 1 and type 2 DM patients with DN (30). Glycated IgG undergoes structural perturbations, compromised complement fixation, and reduced

interaction with complementary antigens (31). Extensive NEG is reported during autoimmune disorders (32). AGEs formed on IgG become targets for circulating antibodies in American Indians with rheumatoid arthritis (32). Anti-IgG-AGE antibodies recognize and bind to glycated IgG, thus preventing the clearance of glycated antibodies by AGE receptors. Other plasma proteins such as albumin, complement C3, fibrinogen, transferrin, haptoglobin, and anti-trypsin were also found to be the targets of NEG. Although proteins with the longest biological half-life are more prone for glycation, glycation of client proteins depends on intrinsic chemical characteristics including charge and localization of target lysine residues and the availability of glyating agents. In general, basic proteins show a greater glycation rate than acidic proteins despite shorter half-life (33).

Receptor for AGEs

AGEs interact with a variety of cell-surface AGE-binding receptors, which ensue their endocytosis and degradation. Alternatively, AGEs trigger cellular activation, pro-oxidant, and pro-inflammatory events. Precise factors that dictate the fate of AGEs are yet to be elucidated. Receptor for AGEs (RAGE) belongs to the immunoglobulin superfamily and express in a wide variety of cells including macrophages, endothelial cells, and neuronal cells. In the kidney, the RAGE protein is found in tubular epithelial cells, glomerular epithelial cells (also known as podocytes), and mesangial cells. RAGE consists of 394 amino acids with a molecular mass of about ~45 kDa (34). Although AGEs were the first identified ligands for RAGE, it is a multi-ligand pattern-recognition receptor. Besides AGEs, other ligands that have been found to interact with RAGE include amyloid β -peptide, amphoterin, and calgranulins. Expression of RAGEs is low under physiological settings but can be up-regulated in response to stress and inflammation. Constitutive expression of RAGE was noticed during embryonic stage; however, in adults, RAGE ligands and inflammatory mediators regulate their expression, with an exception in lungs, where RAGE constitutively expresses in high levels (35). RAGE expression is up-regulated during DN (36). RAGE-transgenic mice develop features of advanced DN such as kidney enlargement, glomerular hypertrophy, mesangial expansion, glomerulosclerosis, and proteinuria (37).

The full-length RAGE consists of three domains: an extracellular domain, a transmembrane domain, and a cytosolic domain (Figure 2B). According to the Human Gene Nomenclature Committee, there are 19 (V1–V19)

naturally occurring splice variants (38). Proteolytic cleavage of full-length RAGE by membrane metalloproteinase (ADAM10) and alternative splicing of RAGE mRNA (RAGE V1) give rise to cleaved RAGE and endogenous secretory RAGE (esRAGE), respectively. Both these RAGE fragments (RAGE V1 and esRAGE) constitute soluble RAGE (sRAGE), which possess only the extracellular domain and is devoid of the transmembrane and cytoplasmic domains. These extracellular RAGEs constitute the predominant species in serum and could act as a decoy receptor that intercepts the interaction of ligands including AGEs with full-length RAGE. Therefore, sRAGE offers natural protection by blocking the interaction of AGEs with full-length RAGE and prevents the activation of adverse signaling events triggered by the AGE-RAGE axis. It should be noted that accelerated accumulation of AGEs, as it happens during diabetes, depletes sRAGE levels, thus compromising the innate anti-AGE defense mechanism.

In contrast to sRAGE, the interaction of AGEs with full-length RAGE activates the AGE-RAGE axis that regulates a variety of cellular events through several intracellular signaling cascades (11, 38, 39). Activated RAGE stimulates the PI3K-PKB- IKK cascade and NF- κB activation and its translocation to the nucleus. NF- κB binds to the RAGE promoter and enhances RAGE expression (38, 40). Thus, the activation of RAGE by AGEs induces RAGE expression. This mechanism could explain the fact that the expression of RAGE is more in those tissues where AGEs accumulate. NF- κB also associates with the promoter region of glyoxalase I and unlike RAGE, it inhibits glyoxalase I expression. Glyoxalase I minimizes AGE levels by catalyzing the conversion of AGE precursors to lactate. Therefore, RAGE activation indirectly contributes to the AGE production via suppressing glyoxalase I expression. Activation of RAGE interrupts an array of cellular events in various cell types. RAGE induces apoptosis of cells by activating p53-Bax expression and by promoting calcium-dependent activation of caspase cascade (38). The interaction of RAGE with its ligands triggers the generation of reactive oxygen species (ROS) via activation of NADPH oxidase. ROS generated during the AGE-RAGE interaction promotes (a) proliferation of cells by promoting JAK-STAT signaling and (b) migration of cells by activating Rho family small GTPases, cdc42, and Rac. Alternatively, activated RAGE promotes cell migration via regulating VCAM-1/AP-1 expression. Activation of RAGE in client cells elicits a pro-inflammatory phenotype and ensues recruitment of inflammatory cells by inducing the secretion of inflammatory cytokines via recruitment of mitogen-activated protein kinases (p38, stress-activated protein kinase and ERK 1/2) or by

downstream activation of the NF- κB cascade. Furthermore, it was shown that the AGE-RAGE axis also elicits autophagy by activating (a) CaMKK β -AMPK and (b) ERK-dependent activation of PIK3C3, a class III member of the PI3 kinase family (38). Taken together, all these manifestations arise as a consequence of the activated AGE-RAGE axis, which propagates cellular dysfunction by a variety of means that may imply in some pathophysiologic conditions including DN.

Besides RAGE, AGEs could also bind to several other cellular binding sites/receptors, including oligosaccharyltransferase-48 (known as AGE-R1), 80 K-H phosphoprotein (known as AGE-R2), galectin-3 (known as AGE-R3), macrophage scavenger receptors, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), fasciclin, EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1/2 (FEEL1/2) and CD36 (38, 39). It remains to be established whether a specific form of AGE preferably binds to a certain type of receptor, or if there is any tissue-specific interaction between ligand and receptor. AGE-R1 expression is decreased in diabetic kidney disease, whereas up-regulation of AGE-R1 augments the removal of AGEs. Mice transgenic for AGE-R1 are protected against the development of DN (41). AGE-R2 gets phosphorylated upon exposure to AGEs. Therefore, it was suggested that it has a role in eliciting AGE signaling in the early stages, and hence termed AGE-R2 (11, 42). Binding of AGEs to AGE-R3 increases its expression, which leads to an increase in AGE-ligand binding and endocytosis by macrophages (11). Elevated glomerular expression of AGE-R3 was noticed in diabetic mice, whereas AGE-R3-deficient diabetic mice suffered from glomerulopathy, mesangial expansion, and proteinuria (41). Together, AGE-R1 and AGE-R3 participate in the detoxification and degradation of AGEs. Cellular removal of AGEs occurs by receptor-mediated endocytosis, after which intracellular degradation of AGEs to low-molecular-weight AGE peptides occurs. These AGE-peptides re-enter the circulation and are filtered across the glomerular filtration apparatus. Two possible fates of these AGE peptides include various degrees of reabsorption by the renal tubule or catabolism by the proximal tubule and excretion in the urine. Free AGE adducts that are bound to individual amino acids are eliminated in the urine with minimal reabsorption in the renal tubules. Effective elimination of AGEs is dependent on normal renal function. An inverse correlation between serum AGE levels and renal function suggests the active participation of the kidney in the excretion of AGEs. Alternatively, it also suggests that the incidence of renal disease is associated with reduced excretion of AGEs. Macrophage scavenger receptor types I and II can bind to AGE

ligands. These receptors, instead of eliciting signal transduction after engagement with AGE ligands, may involve in the detoxification and clearance of AGEs (43). The AGE ligands increase LOX-1 expression in diabetic rats and also interfere with class B type I scavenger receptor for uptake of HDL cholesterol (26).

AGEs and diabetic nephropathy

Both clinical and experimental studies in type 1 and type 2 diabetes strongly suggest AGE involvement in the pathogenesis of diabetic complications including DN. DN is the most common microvascular chronic complication and develops in 20%–40% of type 1 and type 2 diabetic patients. DN accounts for approximately one-third of all new cases of end-stage renal disease (ESRD). Glomerular capillaries that are affected during DM ensue a progressive decline in glomerular filtration rate, accompanied by several stages of proteinuria such as microalbuminuria, macroalbuminuria and overt proteinuria, and culminate in ESRD warranting renal replacement. Progression to ESRD is further enhanced by hyperglycemia and hypertension, which are other features of type 2 diabetes. Renal disease in diabetic patients is characterized by both hemodynamic (hyperfiltration) and structural abnormalities (glomerulosclerosis, interstitial fibrosis). Prominent glomerular changes include thickening of basement membranes, mesangial expansion and hypertrophy and loss of podocytes. Thickening of tubular basement membranes, tubular atrophy, interstitial fibrosis, and arteriosclerosis are prominent changes in the tubulointerstitial compartment during DN. The critical role of AGEs in the pathogenesis of renal damage has been evidenced by a study in nondiabetic rats wherein administration of AGEs induced proteinuria and degenerative changes that were observed in DN (44).

In type 1 diabetic patients, advance from normal renal function to ESRD have significantly increased serum levels of fluorescent non-CML AGEs (45). In contrast, another study revealed that CML in type 1 diabetic patients correlates with the severity of nephropathy (46). In type 2 diabetic patients, there is a significant increase in CML- and hydroimidazolone-AGEs (47). CML-human serum protein levels were higher in those patients with proteinuria and increase in circulating AGE peptides correlated with the severity of renal impairment (41). The severity of DN correlates to the extent of AGE formation and RAGE expression in glomerular and tubulointerstitial compartments. The course of DN is further characterized by decreased

glomerular filtration rate (GFR). AGEs induce asymmetric dimethylarginine (ADMA) in renal tubular cell production, which is an endogenous inhibitor of nitric oxide (NO) synthase. Prevention of renal NO production by AGEs is implicated in the decreased GFR (48).

AGEs alter the metabolism of ECM components and manifest in DN

Accumulation of ECM in the mesangium, tubular interstitium, and glomerular basement membrane (GBM) are hallmark features of DN. AGEs deregulate the balance between synthesis and degradation of ECM components, particularly collagen. Collagen is one of the extensively studied proteins for modification with glycation. AGE formation ensues intra and intermolecular crosslinks in collagen that lead to significant structural changes, including altered packing density and surface charge, together manifested in reduced solubility (15, 49). Glycated collagen compromises its self-assembly and interaction with sub-ECM components. Impaired intermolecular interactions within the collagen fibers and with other components of basement membranes ensue decreased elasticity of arterial vessel walls and capillaries in the glomeruli. Apart from affecting the metabolism of collagen, AGEs alter the interactions among ECM components. Besides, intra-ECM interactions, cell-matrix interactions may also be disrupted by glycation, contributing to impaired cell growth, altered cellular adhesion, and loss of the epithelial phenotype. Modification of various ECM proteins with AGEs impairs their degradation by matrix metalloproteinases, contributing to thickening of the basement membrane and mesangial expansion (50).

The ECM of podocytes and renal capillaries are negatively charged and are able to prevent loss of serum albumin into the glomerular filtrate owing to charge-charge repulsion. However, glycation and accumulation of AGEs in the ECM components of renal capillaries alter the electrostatic interactions that ensue vascular permeability to albumin. AGEs accumulated in ECM components could trap plasma proteins, lipoproteins, and immunoglobulins and affect the homeostasis of normal renal function and aggravate glomerulosclerosis (50). Glycated collagen from the basement membrane increases aggregation propensity of platelets (51). Adverse effects of AGE accumulation on ECM components are also evidenced by studies that employed AGE breakers. Cleavage of AGE-induced crosslinks by ALT-711 and N-phenacylthiazolium

bromide improved the solubility of collagen and reduced matrix accumulation in the kidney (52). AGEs stimulate the expression of transforming growth factor β (TGF- β) in podocytes, tubular cells, and mesangial cells. TGF- β is a pro-fibrotic factor that augments the production of type IV collagen, laminin, and fibronectin. AGEs also induce platelet-derived growth factor, which is also pro-fibrotic. Besides hyperglycemia, glycooxidation and lipoxidation products also contribute to AGEs, including CML, pentosidine, and malondialdehyde-lysine. Altogether, AGEs directly and indirectly alter the metabolism and expression of ECM components that manifest in an advanced nephropathy phenotype (Figure 3).

AGEs induce renal inflammation and fibrosis

Infiltrating macrophages also compounds to disproportionate synthesis and altered metabolism of ECM components during DN. AGEs stimulate monocyte chemoattract protein-1 (MCP-1) expression in mesangial cells, which is associated with monocyte/macrophage infiltration into the mesangium (53, 54). Plasma MCP-1 levels were associated with the rate of albumin excretion. Urinary MCP-1/creatinine ratios in diabetic patients with microalbuminuria were much higher than those in healthy controls

(55). Selective targeting of MCP-1 significantly improved fibrosis and albuminuria in streptozotocin-induced DN (56), suggesting that AGE accumulation in the glomeruli could be implicated in inflammatory and fibrogenic reactions. Another prominent feature of DN is tubulointerstitial injury, which correlates with a decline in renal function. Activation of RAGE induces the expression of TGF- β and other cytokines that are suggested to mediate the epithelial-myofibroblast transdifferentiation of renal tubular cells (57). Exposure to AGEs induced dose-dependent epithelial-myofibroblast transdifferentiation of tubular epithelial cells as determined by morphological changes, alpha-smooth-muscle actin expression, and loss of epithelial cadherin (E-cadherin) expression. Epithelial-myofibroblast transdifferentiation was apparent in the proximal tubules of diabetic rats and a kidney biopsy from a diabetic patient (57).

AGEs induce podocytopathy and mesangiopathy

Glomerular podocytes offer a size-selective filtration barrier that restricts the entry of plasma proteins from the circulation into the urine. Studies in both diabetic subjects and animal models of DN revealed that proteinuria is associated with decreased density of podocytes (58).

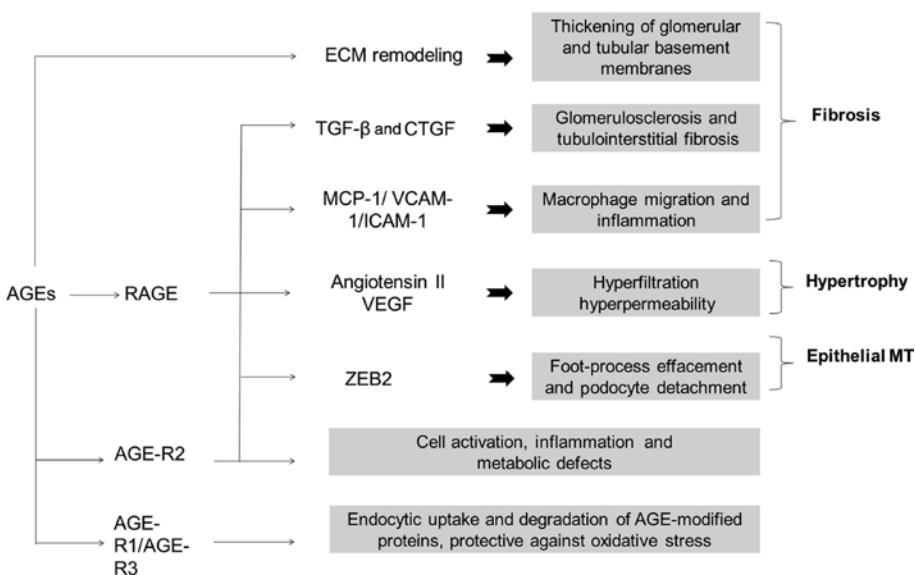


Figure 3: AGE modification of ECM proteins results in remodeling and thickening of the basement membrane.

Stimulation of RAGE by AGEs in addition to increased production of reactive oxygen species results in the activation of various signaling events and the secretion of inflammatory cytokines and growth factors and culminates in several histological and hemodynamic changes implicated in the pathology of DN. Interaction of AGEs with AGE-R2 leads to adverse effects on cells such as inflammation, cell activation and altered metabolic effects, whereas the interaction of AGEs with AGE-R1/R3 results in cellular uptake and degradation of AGE-modified client proteins.

Renal biopsies from patients with DN suggested that AGE accumulation was predominant in the GBM and its accumulation involves up-regulation of RAGE in podocytes (59). In a recent study, we demonstrated that CML activates RAGE expression in podocytes and activates NF- κ B signaling, which in turn induces the expression of zinc finger E-box binding homeobox 2 (ZEB2) (60). ZEB2 is a transcription factor that orchestrates epithelial-mesenchymal transition (EMT) by promoting a 'cadherin switch', which involves suppression of E-cadherin (epithelial marker) and activation of N-cadherin (a mesenchymal marker). EMT of podocytes is implicated in their detachment from GBM, thus decreasing podocyte count in the glomeruli of diabetic rats. On the other hand, exposure of podocytes to CML is associated with loss of placental cadherin (P-cadherin), a key protein localized to the podocyte slit-diaphragm (60). Slit-diaphragm is the size-selective filtration barrier that regulates podocyte perm-selectivity and impedes the loss of high-molecular-weight molecules into the urine. Both CML-induced EMT and loss of P-cadherin expression could be linked to albuminuria in diabetic conditions (Figure 4). It was shown that an activated AGE-RAGE axis ensues TGF- β -induced EMT in normal rat kidney epithelial cells. Mesangial cells play a critical role

in maintaining the integrity of glomerular capillary tufts, whereas AGEs-induced apoptosis of these cells may contribute to glomerular hyperfiltration, an early manifestation of renal dysfunction during DN.

AGEs and the renin-angiotensin system

Renin-angiotensin system (RAS) comprises renin, angiotensinogen, angiotensin I, angiotensin-converting enzyme (ACE), angiotensin II and their receptors. The kidneys synthesize all the components of RAS and the predominant function of RAS is to regulate blood pressure and fluid balance. Activation of the local RAS occurs in the proximal tubular epithelial cells, mesangial cells, and podocytes during DN (61). Renal angiotensin II activity is augmented during the early stages of DN. Angiotensin II is a potent vasoconstrictor, providing a possible explanation for increased renal filtration in the early stages of DN. Inhibitors of ACE, which converts angiotensin I to angiotensin II, decrease renal hyperfiltration and prevent glomerular injury. Angiotensin II triggers hypertrophy of both mesangial and tubular epithelial cells (62, 63). Angiotensin II elicits its action via angiotensin II type 1 receptor (AT1R), whereas AGEs stimulate AT1R expression. Therefore, AGEs are implicated in enhanced angiotensin II activity and a series of adverse events and degenerative changes associated with DN. It was also reported that RAS induces MCP-1 and TGF- β (62–65). MCP-1 promotes macrophage recruitment, thus ensuing extracellular matrix accumulation and renal fibrosis. It was also reported that angiotensin II induces ROS production, which may contribute to podocyte autophagy (66). Our earlier study revealed that accumulation of glomerular CML is associated with elevated expression of MCP-1 and proteinuria in diabetic rats (67).

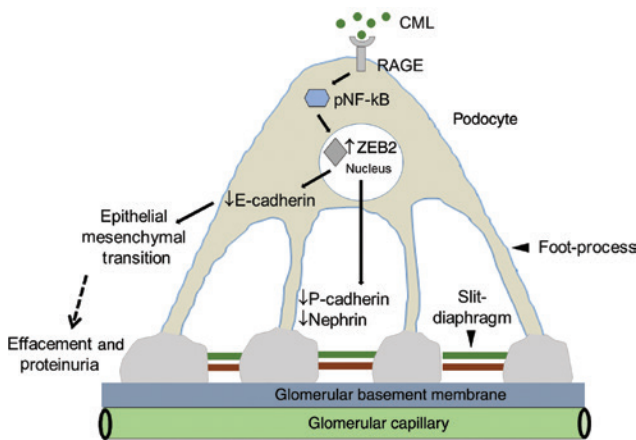


Figure 4: The mechanism by which CML induces podocyte injury and proteinuria.

CML, via RAGE, induces NF- κ B activation, which in turn stimulates ZEB2 expression. Elevated ZEB2 expression orchestrates podocyte damage by two different ways: (1) ZEB2 transcriptionally suppresses E-cadherin expression by which podocytes may undergo epithelial-mesenchymal transition and detach from the basement membrane, which manifests in decreased podocyte count per glomeruli. (2) ZEB2 also suppresses P-cadherin expression, which is localized to slit-diaphragm (SD) protein. Reduced P-cadherin expression ensues in compromised function of SD. CML also suppresses nephrin, another key protein of SD by an unknown mechanism. Together, the loss of E- and P-cadherin and nephrin manifest in decreased podocyte count and proteinuria.

Anti-glycation strategies

The normalization of blood glucose levels in diabetic patients is challenging. Intensive treatment strategies to reduce hyperglycemia in diabetic patients are associated with severe hypoglycemia and increased mortality risk. Although strict control of blood glucose levels inhibits the progression of albuminuria levels, it may not offer significant differences in the outcome of clinical kidney disease. Therefore, to prevent diabetic renal complications, additional therapeutic options are required than

that prescribed for normalization of blood glucose. The steady-state abundance of AGEs reflects not only on the status of glycemia, but also on the balance of oral intake, endogenous formation and their degradation. A natural protective mechanism against deleterious post-translational modifications including glycation is protein turnover (68). Proteins with shorter half-lives are prone to rapid degradation and are minimally affected with glycation. Another natural AGE clearing system includes sRAGE, which binds with AGEs and facilitates their degradation. Considering the adverse events and pathologies associated with AGE accumulation, several natural and synthetic compounds have been screened for their anti-AGE effects (Table 2). Anti-glycation compounds are broadly classified as 1) anti-AGEs that prevent AGE formation, 2) AGE breakers that break preformed AGEs and Amadori products, 3) RAGE inhibitors, 4) redox regulators, and 5) nutraceuticals that serve as anti-glycating agents.

Anti-AGEs

Aminoguanidine (AG) was the first synthetic inhibitor that served as a nucleophilic trap for reactive carbonyl

intermediates, thus preventing the formation of AGEs (69). Administration of AG was effective in improving AGE-mediated diabetic complications by preventing the formation of glomerular microvascular lesions in diabetic rats (26). In addition to inhibiting AGE formation, AG inhibits inducible nitric oxide synthase (iNOS) and prevents accumulation of NO in the diabetic milieu. Clinical trials reported that AG has also shown to improve renal function in diabetic subjects (70). Nevertheless, AG was not approved for therapeutic use owing to its side effects as revealed by phase III clinical trials. Another synthetic anti-AGE compound, 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanidide (OPB-9195), is designed to trap reactive carbonyl intermediates. Treatment with OPB-9195 prevented several phenotypic manifestations of DN and proteinuria that developed in RAGE-transgenic mice (37). This study strongly suggests the involvement of RAGE in the pathogenesis of DN and the AGE-RAGE axis as a promising target for overcoming pathogenesis of DN. Administration of OPB-9195 to hypertensive rats lowered glycosylated albumin levels and increased urinary NO excretion (71). Tenilsetam and 2,3-diamino-phenazine are a couple of inhibitors that act in similar fashion to AG to scavenge the reactive carbonyl intermediates (72). It was reported that

Table 2: List of various anti-AGE compounds that are employed to inhibit or decelerate AGE formation, destabilize preformed AGEs and regulate AGE receptor expression.

Category	Compounds	Mechanism of action	Reference
Anti-AGEs	Aminoguanidine 2,3-diamino-phenazine Tenilsetam OPB-9195 Pyridoxamine Arginine	Traps reactive carbonyl intermediates, scavenges reactive oxygen/nitrogen species; inhibit post-Amadori stages of AGE formation Arginine react with reactive carbonyls and spare the arginine-residue of the proteins from NEG Break Maillard crosslinks	(26, 37, 72, 73) (80, 109)
AGE breakers	ALT-711 ALT-946 PTB Alagebrium		
AGE-detoxifying enzymes	Glyoxalase (I, II and III) Fructosyl-amine oxidase and fructosyl-amine kinase	Converts reactive carbonyls to lactic acid Destabilize Amadori products	(75, 76, 78)
RAGE inhibitors	Anti-RAGE antibodies Pravastatin, curcumin	Neutralize RAGE and prevent cellular activation; inhibit RAGE expression	(81, 86, 110)
Redox regulators	Aldose reductase inhibitors ACE inhibitors and Angiotensin II receptor blockers	Replenish glutathione levels by preventing NADPH consumption during sorbitol pathway; mitigate redox stress and prevent AGEs formation Prevent formation of reactive carbonyl precursor and prevent AGEs formation	(83–85, 89, 90)
Antioxidants	Vitamin C, vitamin E α -Tocopherol, taurine. Niacinamide, oxerutin pyridoxal, lipoic acid, N-acetylcysteine, epigallocatechin-3-gallate	Quench free radicals and deceleration of AGE formation	(91–95, 99)

pyridoxamine traps reactive carbonyl intermediates, scavenges ROS, and also inhibits post-Amadori stages of AGE formation (73). Oral administration of pyridoxamine to diabetic rats attenuated CML accumulation. Pyridoxamine was effective in treating DN as evidenced by phase II clinical trials. Dietary supplementation of arginine could be employed as an anti-AGE agent as exogenous arginine would be modified by reactive carbonyls and spare the innate arginine residue of the proteins from NEG. The development of specific carbonyl stress inhibitors may offer new therapeutic opportunities.

AGE breakers

AGE breakers include AGE-detoxifying enzymes and small chemical molecules that are able to recognize and break the Amadori products or AGE crosslinks. Cells have developed a couple of intracellular detoxifying pathways to prevent the accumulation of AGEs. The glyoxalase system comprises glutathione-dependent enzymes glyoxalase I, II, and III (74, 75). These enzymes catalyze the conversion of carbonyl compounds (glyoxal, methylglyoxal, and α -oxoaldehydes) to lactic acid. Elevated expression of glyoxalase abated hyperglycemia-induced intracellular formation of AGEs, which in turn reduced intracellular oxidative stress and apoptosis of the mesangial cell (75). Another enzymatic system includes fructosyl-amine oxidases and fructosyl-amine kinases, which phosphorylate and destabilize Amadori products, thus ensuing their breakdown (76–78). Fructosamine-3-kinase is one of the most studied enzymes of this AGE detoxification system (78, 79). Both the glyoxalase system and fructosyl-amine oxidase/kinase system confer protection against glycation by preventing the accumulation of highly reactive intermediates of NEG.

Small synthetic molecules such as N-(2-acetamidoethyl) hydrazine carboximidamide hydrochloride (ALT-946), 4,5-dimethyl-3-phenacylthiozolium chloride (ALT-711), and N-phenacetylthiazolium bromide (PTB) have been developed to chemically break Maillard reaction crosslinks. Diabetic rats treated with ALT-711 prevented the glycation of collagen, which is evidenced by reduced collagen III and increased collagen solubility (80). Administration of AGE breaker alagebrium reduced RAGE and AGE-R3 (galectin-3) mRNA expression in diabetic rats suggested the pharmacological potential of these molecules in preventing AGE-mediated diabetic complications (80). ALT-711 reduced tubular AGEs and TGF- β expression and prevented epithelial-myofibroblast transdifferentiation in diabetic rats suggesting that AGE crosslink breakers

are possible treatment options to target tubulointerstitial fibrosis in DN (57).

RAGE inhibitors

As AGEs elicit their effects via interacting with RAGE and also an accumulation of AGEs associated with the overexpression of RAGE, employing RAGE inhibitors could be a potential strategy to combat AGE-mediated adverse cellular manifestations. Anti-RAGE neutralizing antibodies offer promising effects by reducing AGE-mediated effects (81). Another possible approach is to suppress RAGE expression using anti-sense RNA against RAGE mRNA. Alternatively, RAGE-mediated downstream signaling events can be targeted using putative small molecular antagonists. As serum concentrations of sRAGE inversely correlate with AGE-induced pathologies, it has been proposed that neutralization of AGEs by decoy receptors of sRAGE could be another approach to prevent toxicity by AGEs.

Redox regulators

Increased oxidative stress is implicated in the pathogenesis of diabetic complications, including DN. Depletion of endogenous antioxidants and the augmented production of ROS impose the oxidative stress. The rate of conversion of reversible Schiff's base and partially reversible Amadori products to AGEs is accelerated under oxidative stress conditions. Further, the formation of reactive carbonyls under oxidative stress conditions may account for elevated AGEs formation. Therefore, regulating redox conditions might offer a therapeutic intervention by preventing AGE formation. Enzymes such as aldehyde dehydrogenase and glyoxalase employ glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) as co-enzymes and detoxify ROS and carbonyl compounds. Glyoxal and methylglyoxal react with GSH and are subsequently detoxified by glyoxalase I and II into lactic acid. NADPH replenishes GSH by increasing GSH reductase activity. Together, NADPH and GSH are essential redox regulators necessary to cope with oxidative stress arising during glycation. Aldose reductase (AR)-mediated sorbitol pathway is activated during diabetes and consumes NADPH for the conversion of glucose to sorbitol (82). Elevated aldose reductase activity depletes GSH levels. Therefore, inhibitors of AR help to prevent NADPH consumption and to replenish GSH. AR inhibitors prevented CML, pentosidine, and imidazolone formation in diabetic subjects and diabetic animal models (83–85). In addition, antioxidants

including lipoic acid, vitamin E, and ubiquinol prevented the demand for GSH by serving as redox regulators and prevented AGE-mediated cellular toxicity.

Statins and ACE inhibitors as anti-AGEs

Pravastatin has been shown to elicit a renoprotective effect in multiple ways. Pravastatin (a) suppresses RAGE expression, (b) prevents AGE-induced apoptosis of tubular cells and protects from tubular damage in DN, (c) inhibits asymmetric dimethylarginine (ADMA) (inhibitor of iNOS) generation in tubular cells, thus regulating NO levels (86). Atorvastatin inhibited the expression of RAGE induced by AGEs in healthy rats (87). Cerivastatin prevented AGE-stimulated increase in VEGF and NF- κ B expressions in microvascular endothelial cells and prevented AGE-induced angiogenesis (88). Both ACE inhibitors (e.g. Ramipril) and angiotensin II receptor blockers have shown to attenuate the production of reactive carbonyl precursors and attenuated AGE accumulation in diabetic animals (89, 90).

Antioxidants

Oxidation steps are critically involved in promoting the Maillard reaction and the formation of Amadori products subsequently to AGEs. Further, the AGE-RAGE interaction has been shown to induce oxidative stress-sensitive transcription factor NF- κ B. Therefore, a lot of emphasis has been directed towards screening antioxidants and metal chelating agents for their anti-glycating activity. Natural antioxidants such as vitamins C and E, α -tocopherol, niacinamide, pyridoxal, lipoic acid and chelating agents such as sodium selenite, selenium yeast, riboflavin, zinc and manganese has been shown to inhibit glycation (77, 91–93). Dietary supplementation of α -lipoic acid prevented glycation of collagen in fructose-fed rats (94). Vitamins C and E and a combination of N-acetylcysteine with taurine and oxerutin were shown to elicit an *in vivo* anti-glycation potential as evidenced by reduced glycation of skin collagen (95). It was reported that N-acetyl cysteine inhibited ROS-dependent NF- κ B activation, thus preventing AGE-mediated aberrant cellular events.

Dietary recommendations

In addition to the AGEs that are formed in the body, AGEs are also obtained from various dietary sources. Dietary

AGEs are highly present in heat-processed foods and are known to contribute to increased oxidative stress and inflammation. The role of dietary AGEs in human health and disease was ignored as it was assumed that they are poorly absorbed. Nevertheless, it was demonstrated that a significant proportion of ingested AGEs are absorbed from food (96). Consumption of AGE-rich diets is associated with the incidence of kidney disease, whereas dietary restriction of AGEs improved kidney dysfunction (97). Dietary AGEs correlate with circulating AGEs (CML and methylglyoxal) and markers of oxidative stress in healthy subjects (98). Increasing evidence suggests that restriction of dietary AGEs help in combating chronic kidney disease. Several dietary sources rich in antioxidants prevent glycation and accumulation of AGEs. Feeding green tea extract prevented AGE-related collagen crosslinking and formation of fluorescent AGEs in C57BL/6 mice (99). Epigallocatechin-3-gallate revealed a promising *in vitro* anti-glycation potential by acting as a reactive carbonyl scavenger and antagonized AGE-induced pro-inflammatory changes (100). Foods high in carbohydrates have the lowest amount of AGEs (19). Processed foods high in protein and fat, such as meat, cheese, and egg yolk are rich sources of AGEs. A diet heavy in AGEs results in proportional elevations in serum AGE levels in diabetic patients. On the other hand, restriction of AGEs in the diet results in a 30%–40% decrease of serum AGE levels in healthy individuals (18, 101). In diabetic subjects, a diet with high AGE content induced an elevated expression of inflammatory mediators including C-reactive protein, NF- κ B, and VCAM-1 compared with a regular diet (102, 103). The excretion of AGEs is suppressed in DN patients compared to healthy controls. The renal clearance of dietary AGEs is impaired in diabetic subjects with renal insufficiency, whereas dietary restriction of AGEs in patients with renal failure improved tissue injury. Therefore, the dietary intake of AGEs might represent an additional risk for AGE toxicity.

Many spices and herbs including curcumin, ginger, cinnamon, and cloves were shown to inhibit glycation *in vitro*, and their anti-glycation potential is associated with their polyphenolic content (100, 104). Naturally occurring flavonoids, such as lutein, quercetin, and rutin were shown to inhibit AGE formation. It was found that curcumin administration combat oxidative stress and inflammation, thus ameliorating DN (105–107). Feeding of cinnamon to diabetic rats prevented CML accumulation in the glomeruli and improved AGE-mediated loss of expression of key podocyte proteins nephrin and podocin. Procyandin-B2, an active component of cinnamon also prevented accumulation of CML in the glomerular region (67). It is interesting to note that cinnamon and

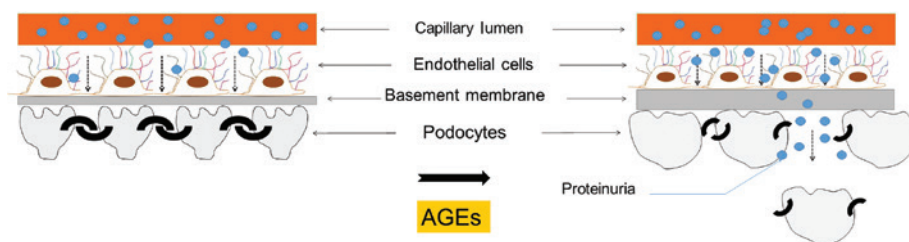


Figure 5: Illustration of the comprehensive effect of AGEs on the glomerular architecture.

AGEs induce thickening of the glomerular basement membrane, hypertrophy of podocytes and podocyte epithelial-mesenchymal transition. These events account for the altered structure of the glomerular filtration barrier and reduced podocyte count that ultimately manifest in proteinuria.

procyanidin-B2 supplementation to diabetic rats exhibited systemic anti-glycation potential as evidenced by a reduction in HbA_{1c} levels and glycation-prone RBC-IgG crosslinks (67). Together, by preventing AGEs in blood and in the kidney, cinnamon and procyanidin-B2 improved proteinuria in diabetic rats. Feeding of ellagic acid (EA), a polyphenol present in fruits and vegetables, to diabetic rats prevented the glycation-mediated RBC-IgG crosslinks and HbA_{1c} accumulation (108). EA ameliorated the accumulation of CML in the kidney and prevented proteinuria in experimental diabetic rats (108). Plant-derived foods like cereals, legumes, vegetables, and fruits that are rich in carbohydrates contain few AGEs unless they are prepared with added fats and exposed to dry-heat cooking methods. By avoiding fatty meats, full-fat dairy products and solid fats dietary, the intake of AGEs can be restricted (19). Dry-heat cooking methods such as broiling, roasting, grilling, baking, sautéing, pan-frying, and deep fat frying propagate and accelerate new AGE formation. AGE formation during cooking can be significantly reduced by cooking food at low temperatures, cooking for short durations, using moist heat methods and by using acidic ingredients like lemon juice.

Conclusion

Considering the magnitude of diabetes incidence, the burden of diabetes on human health and the importance of renal function, it is important to have potent treatment strategies to prevent DN. The dearth of affordable and effective renal care for subjects with chronic kidney disease is also contributing to the global healthcare burden. Proteinuria is also a strong predictor of other diseases, including cardiovascular and cerebrovascular complications. Regulation of glucose homeostasis has been a major treatment option for diabetics. The accumulated evidence argues for the critical role for AGEs in the

pathophysiology of DN as evidenced by the involvement of AGEs in hypertrophy, thickening of the GBM and EMT of podocytes (Figure 5). Therefore, evaluation of the efficacy and safety of anti-AGE compounds including AGE blockers/crosslink breakers, ACE inhibitors, sRAGE and inhibitors of AT1R is the choice of preventive medicine for combating DN. Successful accomplishment of anti-AGE therapy could be a part of a regimen for subjects with diabetic renal complications. Discovering and developing a novel drug target against AGE-mediated renal pathology will be an effective therapeutic intervention against DN. It is evident that both dietary AGEs and local AGEs contribute to tissue injury and impaired renal function. A regimen besides regulating blood glucose to the normal range, antioxidants and anti-AGE compounds in the diet protects from AGE-mediated complications and improve renal function, particularly in diabetics.

Acknowledgments: This work is supported by grants from the Life Science Research Board, DRDO (AKP); Department of Science and Technology, Department of Biotechnology and ICMR (GBR), Government of India. As this article is focused on AGEs and diabetic nephropathy, some interesting publications in the field of non-enzymatic glycation could not be mentioned herein.

Conflict of interest statement: The authors declare no conflicts of interest in this work.

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