Advanced Glycation End Products: Sparking the Development of Diabetic Vascular Injury

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Advanced Glycation End Products
Sparking the Development of Diabetic Vascular Injury

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Abstract—Advanced glycation end products (AGEs) are proteins or lipids that become glycated after exposure to sugars. AGEs are prevalent in the diabetic vasculature and contribute to the development of atherosclerosis. The presence and accumulation of AGEs in many different cell types affect extracellular and intracellular structure and function. AGEs contribute to a variety of microvascular and macrovascular complications through the formation of cross-links between molecules in the basement membrane of the extracellular matrix and by engaging the receptor for advanced glycation end products (RAGE). Activation of RAGE by AGEs causes upregulation of the transcription factor nuclear factor-κB and its target genes. Soluble AGEs activate monocytes, and AGEs in the basement membrane inhibit monocyte migration. AGE-bound RAGE increases endothelial permeability to macromolecules. AGEs block nitric oxide activity in the endothelium and cause the production of reactive oxygen species. Because of the emerging evidence about the adverse effects of AGEs on the vasculature of patients with diabetes, a number of different therapies to inhibit AGEs are under investigation. **(*Circulation*. 2006;114:597-605.)**

Key Words: advanced glycosylation end products ■ diabetes mellitus ■ vasculature

Advanced glycation end products (AGEs) are modifications of proteins or lipids that become nonenzymatically glycated and oxidized after contact with aldose sugars.[1,2] Early glycation and oxidation processes result in the formation of Schiff bases and Amadori products. Further glycation of proteins and lipids causes molecular rearrangements that lead to the generation of AGEs.[1] AGEs may fluoresce, produce reactive oxygen species (ROS), bind to specific cell surface receptors, and form cross-links.[1,3] AGEs form in vivo in hyperglycemic environments and during aging and contribute to the pathophysiology of vascular disease in diabetes.[4–7] This review summarizes AGE formation and biochemistry, cellular receptors for AGE, AGE-induced effects on extracellular and intracellular functions, and developing AGE therapies.

AGEs accumulate in the vessel wall, where they may perturb cell structure and function. AGEs have been implicated in both the microvascular and macrovascular complications of diabetes. As reviewed by Brownlee,[5] AGEs may modify the extracellular matrix (ECM); modify the action of hormones, cytokines, and free radicals via engagement of cell surface receptors; and impact the function of intracellular proteins.

AGE Biochemistry

Key factors crucial to the formation of AGEs include the rate of turnover of proteins for glycoxidation, the degree of hyperglycemia, and the extent of oxidant stress in the environment.[1,5,8–10] If one or more of these conditions is present, both intracellular and extracellular proteins may be glycated and oxidized. The AGE formation process, or the Maillard reaction, begins from Schiff bases and the Amadori product, a 1-amino-1-deoxyketose, produced by the reaction of the carbonyl group of a reducing sugar, like glucose, with proteins, lipids, and nucleic acid amino groups.[5,11] During Amadori reorganization, these highly reactive intermediate carbonyl groups, known as α-dicarbonyls or oxoaldehydes, products of which include 3-deoxyglucosone and methylglyoxal, accumulate.[12,13] Such buildup is referred to as "carbonyl stress." The α-dicarbonyls have the ability to react with amino, sulfhydryl, and guanidine functional groups in proteins.[14] The reaction results in denaturation, browning, and cross-linking of the targeted proteins.[14,15] In addition, the α-dicarbonyls can react with lysine and arginine functional groups on proteins, leading to the formation of stable AGE compounds, such as N^{ϵ}-(carboxymethyl)lysine (CML), which are nonfluorescent AGEs.[16] CMLs also form in vitro from LDL incubated with copper ions and glucose and therefore are believed to be both lipid and protein adducts.[17,18] Once AGEs are formed, they are nearly irreversible.[8] There is evidence that enzymes, such as glyoxalase-1, have the ability to detoxify AGE precursors and inhibit AGE production, as evidenced by the presence of deoxyfructose, a reduction product of 3-deoxyglucosone in human urine and plasma.[5,19]

Receptors for AGEs

Several different receptors for AGEs have been discovered, one of which, termed RAGE, initiates the intracellular sig-

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naling that disrupts cellular function through its recognition and binding of AGEs. RAGE is a member of the immunoglobulin superfamily of receptors.[20,21] The human *RAGE* gene is on chromosome 6 in the major histocompatibility complex between genes for class II and class III.[22] Nuclear factor (NF)-κB sites, an interferon-γ response element, and an NF–interleukin-6 (IL-6) DNA binding motif are located on the RAGE promoter.[23] NF-κB sites control cellular expression of RAGE, linking RAGE to the inflammatory response.[23] RAGE has a 332–amino acid extracellular component, consisting of 2 "C"-type domains preceded by 1 "V"-type immunoglobulin-like domain.[24–26] RAGE has a single transmembrane domain followed by a highly charged 43–amino acid cytosolic tail.[26] The V domain in the *N*-terminus is important in ligand binding, and the cytosolic tail is critical for RAGE-induced intracellular signaling.[26] A form of RAGE that lacks the cytosolic tail but stays embedded in the membrane where it binds AGEs functions as a dominant-negative RAGE, unable to transduce a cell signal on ligand engagement.[26,27]

RAGE may be complexed with another polypeptide, termed LF-L, for its likeness to lactoferrin, at least in certain cell types.[28] LF-L can bind AGEs and can also bind noncovalently to the extracellular domain of RAGE.[28] RAGE is minimally expressed in normal tissue and vasculature.[26] However, RAGE is upregulated when AGE ligands accumulate, an example of positive-feedback activation.[21] Upregulation of RAGE occurs on cells such as endothelial cells, smooth muscle cells, and mononuclear phagocytes in diabetic vasculature.[26,29] In diabetic vessels, RAGE ligands include AGEs of at least 2 varieties: CML adducts and hydroimidazolones.[25,30] CML-AGEs are the most prevalent AGEs in vivo.[31] CML adducts are signal-transducing ligands for RAGE, both in vitro and in vivo.[25] Hydroimidazolone AGEs are derived from methylglyoxal and 3-deoxyglucosone.[32]

Other receptors, like AGE-R1 (oligosaccharyl transferase-48), -R2 (80K-H phosphoprotein), and -R3 (galectin-3), and the class A macrophage scavenger receptor types I and II, also are able to recognize and bind AGE ligands, but they have not been shown to transduce cellular signals after engagement by AGEs.[33,34] Instead, they may cause the clearance and possible detoxification of AGEs.[34] AGE-R1 is a type 1 single transmembrane integral protein. AGE-R1 has a small extracellular *N*-terminal domain and a cytoplasmic *C*-terminal domain.[33,35–37] AGE-R2 is an 80- to 90-kD protein involved in the intracellular signaling of various receptors, like the fibroblast growth factor receptor.[33,38] AGE-R2 contains a tyrosine-phosphorylated section in the plasma membrane of the cell.[38,39] AGE-R3, whose binding domain is at the *C*-terminus, also binds AGE ligands with high affinity.[33,40] Two class B scavenger receptors, CD36 and class B type I, also bind AGE ligands. CD36 is not involved in the clearance of AGEs from the circulation, but it plays an important role in the induction of oxidative stress in the cell.[41,42] AGE ligands interfere with scavenger receptor class B type I uptake of HDL cholesterol.[43,44] AGEs bind to and are recognized by the class E scavenger receptor, lectin-like oxidized LDL receptor-1 (LOX-1), and AGEs increase LOX-1 expression in diabetic rats.[45,46] Fasciclin, epidermal growth factor–like, laminin-type epidermal growth factor–like, and link domain–containing scavenger receptor-1 and -2 (FEEL-1 and FEEL-2) also are scavenger receptors that bind AGEs.[47]

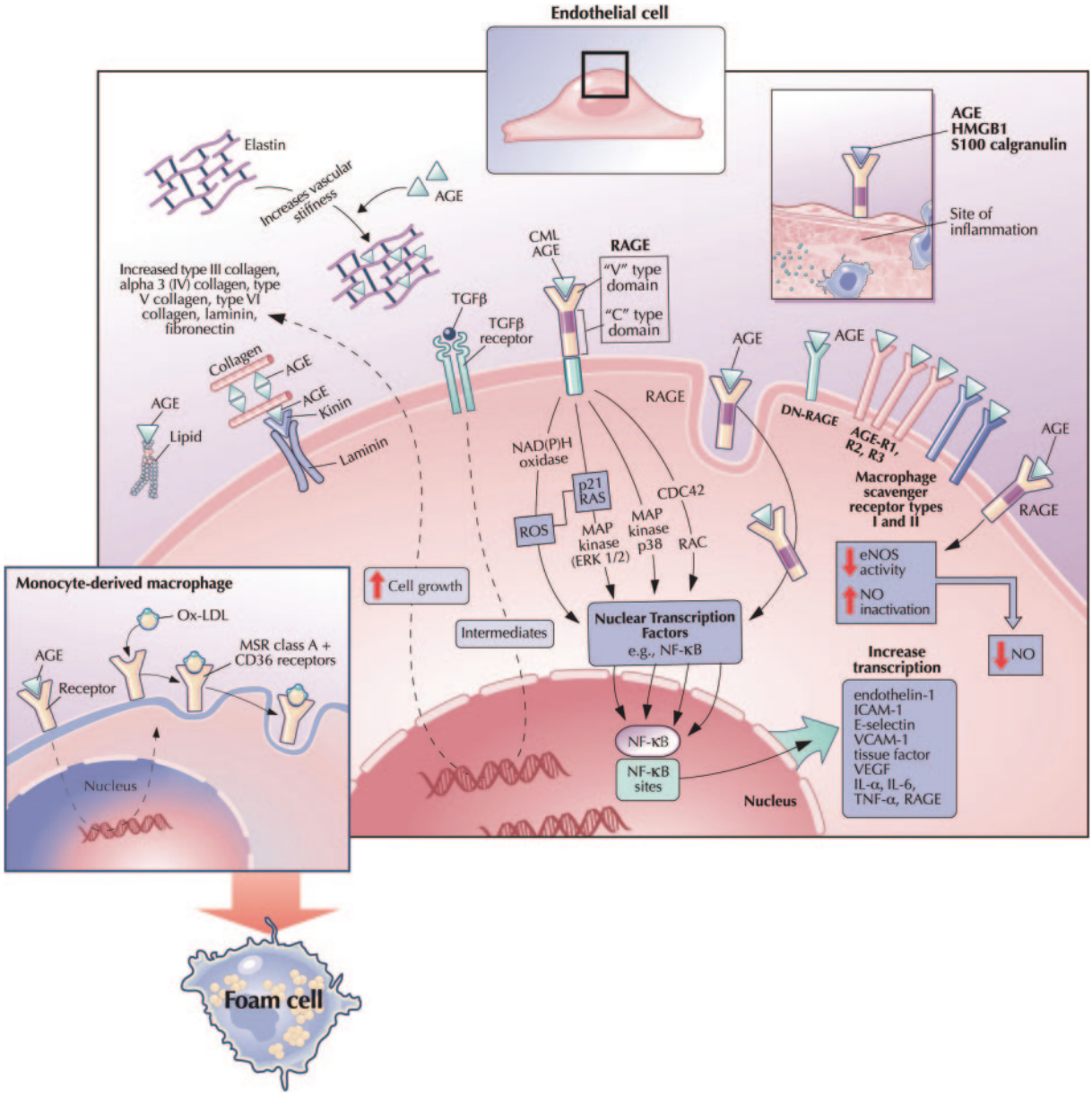
AGE Effects on Extracellular Function

General mechanisms through which AGEs contribute to diabetic complications include the following: (1) formation of cross-links between key molecules in the basement membrane of the ECM, permanently altering cellular structure; and (2) interaction of AGEs with RAGE on cell surfaces, altering cellular function (the Figure).

Formation of AGEs in the ECM occurs on proteins with a slow turnover rate. Accumulation of AGEs on proteins in the ECM can cause formation of cross-links, which can "trap" other local macromolecules.[9,48] AGEs can alter properties of the large matrix proteins collagen, vitronectin, and laminin, through AGE-AGE intermolecular covalent bonds, or cross-linking.[48–50] AGE cross-linking on type I collagen and elastin causes an increase in the area of ECM, resulting in increased stiffness of the vasculature.[5,11,51–53] Glycation results in increased synthesis of type III collagen, α3(IV) collagen, type V collagen, type VI collagen, laminin, and fibronectin in the ECM, most likely via upregulation of a transforming growth factor-β intermediate.[54–57] AGEs disrupt binding of the noncollagenous domain (NC-1) to the helix-rich domain on type IV collagen from the basement membrane, inhibiting the formation of a matrix-like structure.[5,58,59] Formation of AGEs on laminin results in reduced binding to type IV collagen, reduced polymer elongation, and reduced binding of heparan sulfate proteoglycan.[60] Glycation of laminin and type I and type IV collagens, key molecules in the basement membrane, causes inhibited adhesion to endothelial cells for both matrix glycoproteins.[51,61] In diabetic subjects, the glycation of these proteins can lead to disparities in production, growth, and secretory activity of different types of cells.[51] Studies suggest that AGE formation leads to a reduction in the binding of collagen and heparan to the adhesive matrix molecule vitronectin.[5] AGE-induced alterations of vitronectin and laminin may explain the reduction in binding of heparan sulfate proteoglycan, a stimulant of other matrix molecules in the vessel wall, to the diabetic basement membrane.[5,49,62] In addition to the formation of AGEs on proteins, AGEs can also form on lipids, as evidenced by the increased lipid-linked AGEs in LDL samples from persons with and without diabetes.[63] Glycated LDL reduces nitric oxide (NO) production and suppresses uptake and clearance of LDL through its receptor on endothelial cells.[63–65]

AGE Effects on Intracellular Function

AGEs also form on intracellular proteins. Intracellular AGEs change cellular properties that are critical in vascular homeostasis.[66] The rate of AGE formation on intracellular proteins is slowest in the presence of glucose and more rapid with intracellular natural sugars, like fructose, glyceraldehyde-3-phopshate, and glucose-6-phosphate.[5,67] Ten times more fructose-derived AGEs form after 5 days than glucose-derived AGEs in vivo.[68] Intracellular AGE formation significantly increases in endothelial cells after 1 week in a



The extracellular and intracellular effects of AGEs. In the ECM, AGEs form on a variety of different molecules, including lipids, collagen, laminin, elastin, and vitronectin. The formation of AGEs on ECM molecules alters the constitution of the matrix and increases stiffness. AGEs also activate the transforming growth factor (TGF)-β receptor to stimulate cell growth, leading to increased ECM production. AGEs that bind to RAGE on the endothelial cell surface lead to a signaling cascade, stimulating NAD(P)H oxidase and increasing ROS, p21 RAS, and MAPKs. In addition, the ligand-RAGE interaction also may stimulate signaling via p38 MAPK and Rac/Cdc. A key target of RAGE signaling is NF-κB. NF-κB is translocated to the nucleus, where it increases transcription of a number of different proteins, including endothelin-1, ICAM-1, E-selectin, and tissue factor. AGE and ligands for RAGE, such as HMGB1 and S100 calgranulins, trigger inflammatory pathways. AGE may decrease NO availability by the decreased activity of NOS and by quenching NO. sAGEs activate monocytes, causing increased expression of macrophage scavenger receptor (MSR) class A receptors and CD36 receptors, leading to increased OxLDL uptake and foam cell formation. DN indicates dominant-negative.

hyperglycemic environment.[69] Intracellularly, basic fibroblast growth factor is one of the proteins that may be glycated.[70] AGE modification of this protein drastically reduces the mitogenic activity of endothelial cell cytosol by 70%.[70]

Circulating AGEs may interact with endothelial RAGEs, which leads to perturbation of cellular properties, such as upregulation of the transcription factor NF-κB.[9,71] Activation of RAGEs by AGEs transduces multiple signals, such as NAD(P)H oxidase, $p21^{ras}$, the mitogen-activated protein kinases (MAPKs), extracellular signal–regulated kinase 1/2 and p38, and the GTPases Cdc42 and Rac, resulting in activation and translocation of nuclear transcription factors, including NF-κB, which transcribes its target genes (the Figure).[72–75] Among these are endothelin-1, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, tissue factor, thrombomodulin, vascular endothelial growth factor (VEGF), and likely, proinflammatory cytokines, including IL-1α, IL-6, and tumor necrosis factor-α, and RAGE itself.[76,77] Blockade of RAGE with anti-RAGE IgG or

soluble RAGE (sRAGE), the extracellular ligand, inhibits NF-κB activation.[8]

AGE and the Endothelial Cell

sAGEs are chemotactic for human blood monocytes both in vitro and in vivo.[78,79] AGEs on the subendothelium induce monocyte migration across an endothelial cell monolayer.[79] In human umbilical vein endothelial cells, inhibitors of NF-κB greatly reduce high glucose–induced monocyte adhesion, suggesting that the activation of NF-κB is essential in AGE-induced monocyte adhesion and migration.[78] Activation of RAGE by AGEs increases endothelial permeability to macromolecules, yet another receptor-mediated effect of AGEs on the diabetic vasculature.[23,71] AGEs located on proteins, in addition to immobilized AGEs on the subendothelium, bind RAGE on the endothelium to induce hyperpermeability.[77] Administration of sRAGE inhibits vascular leakage in the intestine and skin of streptozotocin-treated rats.[8] AGE-bound RAGE on the endothelium also results in alteration of the cell surface structure, from that of an anticoagulant to a procoagulant endothelium, via reduced thrombomodulin activity concomitant with increased tissue factor expression.[71,77,80]

AGE and the Macrophage

Whereas sAGEs activate monocytes, AGEs located in basement membranes inhibit monocyte migration, inducing a process called "apoptaxis." AGEs may contribute to the expression of oxidized LDL (OxLDL) receptors in human monocyte–derived macrophages.[81] AGEs have been shown to induce gene expression of 2 important OxLDL receptors: macrophage scavenger receptor class A and CD36.[81] The increased expression of these receptors leads to enhanced OxLDL uptake, resulting in foam cell transformation (the Figure). Activation of monocytes by AGE-modified human serum albumin also leads to expression of IL-1β and tumor necrosis factor-α mRNA.[82] AGEs also alter cellular coagulant properties, partly via the monocyte-produced procoagulant, tissue factor, and decreased expression of the endothelial anticoagulant cofactor, thrombomodulin.[23,71,80,83,84]

AGE and the Smooth Muscle Cell

Addition of AGE-albumin to rat pulmonary artery smooth muscle cells results in increased levels of GTP-bound $p21^{ras}$ and activation of ERK1 and ERK2 (ie, MAPKs), whereas addition of nonglycated albumin to the same type of cells yields basal levels of GTP-bound $p21^{ras}$ and nonactivated MAPKs.[85] $p21^{ras}$ is critical for signal transduction of AGEs, as evident when Cys^{118}, a molecular target of ROS on $p21^{ras}$, is mutated and overexpressed in PC12 cells expressing RAGE. The mutated PC12 cells are nonresponsive to AGE-albumin, whereas the wild-type cells respond to AGE-albumin by activating ERK 1/2 kinases.[85]

AGE Effects on NO

AGEs reduce the bioavailability and activity of endothelium-derived NO (Figure). Because NO inhibits many of the mechanisms that contribute to atherosclerosis, such as leukocyte adhesion to the vessel wall, vascular smooth muscle growth, and platelet adhesion and aggregation, this effect of AGEs on NO may be relevant to atherogenesis.[67,86,87] Indeed, Hogan et al[68] have demonstrated that matrix-bound and sAGEs inhibit the antiproliferative effects of NO. Moreover, impaired vasodilation in diabetes may be a result of AGEs' reduction of NO activity.[67] The levels of serum AGEs in patients with type 2 diabetes are inversely related to the degree of endothelium-dependent and endothelium-independent vasodilation.[88] Several mechanisms by which AGEs reduce or block NO activity have been proposed. One mechanism suggests that AGEs reduce the half-life of endothelial NO synthase (eNOS) mRNA through an increased rate of mRNA degradation and reduced eNOS activity.[89] Another mechanism proposes that AGEs impair NO production via the binding of CML residues to endothelial AGE receptors, causing a reduction in phosphorylation of serine residues in eNOS, resulting in deactivation of the enzyme.[90,91] AGEs also may quench and inactivate endothelium-derived NO.[67,68] Also, the endothelial production of prostacyclin (PGI_2) is reduced by AGEs.[92] In addition to affecting the activity of these 2 major vasodilators, AGEs also enhance the expression of endothelin-1, via NF-κB, in bovine aortic endothelial cells incubated with erythrocytes from patients with type 2 diabetes.[93]

AGE-bound RAGE in the endothelium results in the production of reactive oxygen intermediates, triggered, at least in part, through the activation of NADPH oxidase.[72,94] AGE-RAGE interaction stimulates the production of reactive oxygen intermediates, which in turn activate a range of signaling pathways, the consequences of which include activation of NF-κB.[72,94,95]

The Specific AGEs: CML-AGEs

AGE-RAGE–produced oxidative stress activates NF-κB and affects the transcriptional activation of numerous cytokines and adhesion molecules, many of which are closely linked to inflammation and atherosclerosis, as discussed earlier.[72,96] Through the appearance of thiobarbituric acid–reactive substances (TBARS) and activation of NF-κB, mononuclear phagocytes are also affected by oxidative processes resulting from the presence of AGEs. In addition, AGEs found on the surface of erythrocytes can bind to RAGE, increasing TBARS levels and activating NF-κB. The source of ROS on diabetic erythrocytes is most likely AGEs bound to the erythrocyte surface, because engagement of RAGE by antibodies does not produce oxidant stress.[97] In mice infused with AGE-albumin, the induction of oxidant stress leads to activation of NF-κB, induction of heme oxygenase mRNA, and TBARS in the tissues.[72]

Anti-AGE Therapies

A variety of different compounds that inhibit AGEs have been under investigation. Aminoguanidine is a hydrazine compound that prevents AGE formation.[72] Aminoguanidine reacts mostly with derivatives of early glycation products that are not bound on proteins, like 3-deoxyglucosone.[72] Several avenues of investigation have suggested that aminoguanidine favorably affects vascular structure in experimental models of diabetes. The reduction of AGE formation by aminoguani-

dine attenuates the effects of diabetes on large arteries.[52] Aminoguanidine treatment increases arterial elasticity as measured by aortic input impedence, static compliance, and left ventricular afterload in diabetic rats.[52] Aminoguanidine reduces ECM accumulation of both fibronectin and laminin in streptozotocin-induced diabetic rats with diabetic nephropathy.[98] In addition, aminoguanidine decreases vascular AGE accumulation and the severity of atherosclerotic plaque in diabetic rats.[99] Aminoguanidine is also an NOS inhibitor, which may offset some of its benefits as an AGE inhibitor.[67,100] In a placebo-controlled, randomized trial in patients with type 1 diabetes mellitus, aminoguanidine caused a slower reduction in glomerular filtration rate. Aminoguanidine reduced 24-hour urinary proteinuria and progression of retinopathy.[101] However, aminoguanidine did not attenuate the time to doubling of serum creatinine.[101]

N-(2-Acetamidoethyl)hydrozinecarboximidamide hydrochloride (ALT-946) has been shown to be an effective inhibitor of AGE-induced cross-links.[102] Like aminoguanidine, ALT-946 also inhibits NOS, though less so than aminoguanidine.[102] Like aminoguanidine, (±)-2-isopropylidenehydrazono-4-oxothiazolidin-5-ylacetanidide (OPB-9195) is constructed to prevent alteration of nucleophilic residues in proteins by trapping carbonyl intermediates.[103] One study has shown that administration of OPB-9195 to hypertensive rats lowered levels of glycated albumin and increased urinary NO excretion and expression of eNOS mRNA when compared with control rats.[104]

4,5-Dimethyl-3-phenacylthiozolium chloride (ALT-711) is a compound that breaks the cross-links of AGEs. It has a thiazolium structure that is able to sever α-carbonyl compounds by breaking the carbon-carbon bonds between carbonyls.[53,103] Diabetic rats treated for 4 months with ALT-711 show reduced collagen III, increased collagen solubility, and reduced RAGE and AGE-R3 mRNA expression compared with placebo.[105] In addition, ALT-711 has been shown to improve left ventricular function, reduce ventricular collagen, and lengthen survival in diabetic animals, including aged animals in whom there was a large reduction of left ventricle chamber stiffness.[106,107] In older humans, ALT-711 is reported to improve arterial compliance and decrease pulse pressure.[53]

Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) not only inhibit cholesterol production but also block synthesis of isoprenoid intermediates that function as "platforms" for several intracellular signaling molecules.[108,109] Cerivastatin prevents AGE-stimulated increases in VEGF mRNA, NF-κB expression, and DNA synthesis in microvascular endothelial cells and also prevents AGE-induced angiogenesis by interfering with the intracellular AGE signal transduction pathway.[109] Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers have been shown to decrease the production of reactive carbonyl precursors.[110] In one study, the ACE inhibitor ramipril attenuated AGE accumulation in diabetic animals.[111]

sRAGE, the extracellular ligand-binding domain of RAGE, blocks AGEs from binding to RAGE, as if sRAGE were a "sponge" soaking up sAGEs.[112] In doing so, sRAGE suppresses accelerated atherosclerotic lesion formation and decreases vascular hyperpermeability.[112–115] Levels of AGEs and RAGE are increased in streptozotocin-treated (diabetic) apolipoprotein E–null mice that have advanced atherosclerosis by 14 weeks of age.[26] Administration of sRAGE suppresses the severity of atherosclerosis to the same level as that found in euglycemic control mice.[26] Blockade of RAGE by sRAGE in mice with diabetes reduces atherosclerotic lesion area, supporting the notion that RAGE is a crucial part of the development and acceleration of atherosclerosis and that sRAGE is an effective intervention.[113,114,116]

Pyridoxamine, the natural form of vitamin B_6, is effective at inhibiting AGEs at 3 different levels.[117–120] Pyridoxamine prevents the degradation of protein-Amadori intermediates to protein-AGE products.[121] In diabetic rats, pyridoxamine reduces hyperlipidemia and prevents AGE formation.[122–124] Pyridoxamine scavenges the carbonyl byproducts of glucose and lipid degradation. Zucker rats treated with pyridoxamine have reduced plasma levels of glyoxal, methylglyoxal, and AGEs in collagen.[125,126] Benfotiamine, a lipid-soluble thiamine derivative, inhibits the AGE formation pathway.[127,128] In endothelial cells, water-soluble thiamine is similar to benfotiamine in inhibiting AGE formation in high-glucose environments.[128]

AGEs can be absorbed through the diet.[129] Foods high in protein and fat, such as meat, cheese, and egg yolk, are rich in AGEs.[130] Foods high in carbohydrates have the lowest amount of AGEs. In addition, increased cooking temperatures, like broiling and frying, and increased cooking times lead to increased amounts of AGEs.[131] A diet heavy in AGEs results in proportional elevations in serum AGE levels and increased AGE cross-linking in patients with diabetes.[129] Conversely, dietary AGE restriction causes a 30% to 40% decrease of serum AGE levels in healthy subjects.[131] Diabetic patients on a high-AGE diet have increased expression and activity of MAPK, NF-κB, and VCAM-1 compared with diabetic subjects on a low-AGE diet.[132] Excretion of AGEs absorbed through the diet is suppressed in diabetic nephropathy patients compared with healthy controls.[129] Patients with diabetes and renal failure who restrict dietary AGE intake demonstrate suppressed AGE-related tissue injury.[130,133]

Summary

AGEs form when proteins or lipids interact with aldose sugars for an extended period of time, subsequently undergoing molecular transformations that glycate the protein or lipid, thereby imparting distinct and likely maladaptive signatures in the vessel wall. In hyperglycemic environments and in natural aging, AGEs alter cell structure and function. The recognition and binding of AGEs to RAGE contribute to the microvascular and macrovascular complications of diabetes. Understanding AGE formation and biochemistry, cellular receptors for AGE, and AGE-induced effects on extracellular and intracellular functions will serve to expedite the process of finding effective therapies that block excessive accumulation of these species and their interaction with the signal transduction receptor RAGE.

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Disclosures

None.

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