The Maillard Reaction in Eye Diseases

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ABSTRACT: Diabetes and age-related eye disorders remain leading causes of blindness worldwide. While defined pathogenic mechanisms for many of these diseases remain elusive, there is increasing evidence that products of the Maillard reaction may play an important role in their etiology. Advanced glycation end products (AGEs) form through a range of pathways within Maillard chemistry, and there is evidence to suggest that these adducts accumulate in the intracellular and/or extracellular environment of ocular structures. This review evaluates the ever-growing literature on AGEs in biological systems and draws relevant links to diseases such as diabetic retinopathy, age-related macular degeneration, and cataract formation. It also outlines recent pharmaceutical strategies to inhibit Maillard reaction products and provides links to how these may serve to limit ocular cell dysfunction.

KEYWORDS: advanced glycation end products; diabetes; age; retinopathy; eye

INTRODUCTION

The Maillard reaction was first described in the context of food science, where its products were discovered to impart changes in food texture, bioavailability, flavor, and preservation. Maillard chemistry is now known to be very relevant in vivo, where it has important implications for health and disease. In the body, the reaction between reducing sugars and/or carbonyls with free amino groups culminates in the formation of advanced glycation end products (AGEs), and these adducts may then accumulate intracellularly and extracellularly on proteins, lipids, and nucleic acids. Over the last 20 years it has become evident that AGE modification represents a major pathogenic factor in aging and in a spectrum of human diseases such as diabetic complications, neurodegeneration (including Alzheimer’s disease), impotence, pulmonary fibrosis, ischemic heart disease, and atherosclerosis. There is also accumulating evidence that AGEs could play an important pathogenic role in seemingly unrelated eye diseases.

The vertebrate eye has evolved to optimize transmittance of light photons to the neural retina according to particular environmental restrictions (terrestrial or aqueous environments). The array of subtle ocular configurations amongst vertebrates reflects the diversity of evolutionary solutions according to particular visual...
requirements. It also exemplifies the reliance of many animals on vision and the range of options for enabling efficient conversion of light into neural transmission. Irrespective of structural differences in the lens, uvea, cornea, retina, and ocular blood supply between animal groups, there is a common requirement to maintain structural integrity, optical clarity, and adequate nourishment for the highly specialized cells of the eye. For example, an opaque lens will prevent light penetration to the retina and reduce visual acuity. Unfortunately, many of the differentiated cells of the mammalian eye have little or no regenerative capacity. This makes these cell structures highly susceptible to aging processes and systemic diseases that alter structural proteins and/or result in metabolic imbalance. Indeed, ophthalmologists and vision scientists have long recognized that the eye is profoundly influenced by diseases such as diabetes and age-related dysfunction, which, together, account for the leading causes of visual impairment worldwide.

This article reviews some of the important effects that Maillard chemistry has on the cells of the eye and the associative or major role that AGEs may play in the initiation and progression of sight-threatening disorders such as diabetic retinopathy, glaucoma, cataract formation, and age-related macular degeneration (AMD). Also considered are potential pharmacologic strategies to prevent or neutralize the effects of AGEs and how these strategies might be employed for eye diseases and age-related dysfunction.

CHEMISTRY OF AGE FORMATION

AGEs can come from many sources. Nonenzymatic Maillard reactions initially result in the formation of a Schiff base between glucose (or other reducing sugars) and the ε-amino group of proteins, lipids, or DNA that slowly rearrange to the relatively stable Amadori adduct. For example, in proteins the ε-amino group may be represented by lysine with the resultant formation of fructose-lysine as a freely reversible Schiff base. Subsequent Amadori adducts are the first stable product formed during glycation of protein with a half-life of several months under physiological conditions. Nevertheless, despite a modicum of stability, Amadori compounds can undergo further oxidation and dehydration reactions, according to presence of metal ions, giving rise to additional protein-bound compounds collectively termed AGEs. Lipid peroxidation reactions can also form a class of Maillard products called advanced lipoxidation end products. Indeed, lipids are important sources of chemical modifications of proteins especially in lipid-rich, highly oxidative environments, such as in the retina, and dyslipidemia may be an important pathogenic force in retinopathies.

Beyond nonenzymatic glycation, it is now appreciated that autooxidation of free sugars, superoxide production, and metabolism of glucose can lead to high levels of reactive dicarbonyls, such as methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG). These dicarbonyls can lead to very rapid AGE formation especially in circumstances of enhanced glycolytic activity (such as in hyperglycemia), although the net importance of AGEs such as imidazolones are not fully understood. Under normal circumstances the cell can protect itself against these dicarbonyls through a range of intracellular detoxifying enzymes that serve to limit adduct formation of important structural and functional proteins. Alterations in these enzymes
during disease may have implications for AGE accumulation and pathogenic effects in cells and tissues. Indeed, it has been demonstrated that upregulation of glyoxalase-1 can reverse high-glucose mediated AGE formation over a short, 10-day period and prevent AGE-mediated cell abnormalities.

Because the products of advanced glycation/lipoxidation reactions are constantly forming under physiological conditions, it has been suggested that complex receptor systems may have evolved to remove senescent, glycation-modified molecules and/or degrade existing AGE/ALE cross-links from tissues, thereby limiting their deleterious effects. Such receptors could play a critical role in AGE-related biology and the pathology associated with diabetes and aging disorders. Several AGE-binding molecules have been described and it is thought that many of the adverse effects caused by advanced glycation are mediated via AGE receptors such as the receptor for AGES (RAGE), AGE-R1, galectin-3, CD36, and the type I and II scavenger receptor. The relative pathogenic contribution of these receptors in instigating diabetic complications is poorly defined, although RAGE is by far the best characterized. Mechanistic in vitro and in vivo studies on RAGE and its regulatory fragments, such as soluble RAGE (sRAGE), indicate an important role pathobiology.

AGEs IN OCULAR TISSUES

Cornea

From the scientific literature it is clear that the pathophysiological significance of Maillard chemistry is best appreciated in macrovascular systems and in diabetic nephropathy. The role of AGEs in eye disease is less well understood, but it is evident that these adducts could have a role to play in some age- and diabetes-related ocular disorders. In the cornea, for example, the Maillard reaction probably plays a significant role in altering structural proteins during diabetes and aging. Diabetic keratopathy has an association with neuropathic disease and is manifested by thickening of the corneal stroma and Descemet's/Bowman's basal laminae, recurrent erosions, corneal edema and morphological abnormalities in the epithelial and endothelial layers. Such alterations in the human diabetic cornea are accompanied by decreased protein stability in the stroma and increased immunoreactive AGEs which have been partially characterized as pentosidine and CML. Bowman's membrane is heavily glycated in diabetic patients while in vitro, AGE-modified substrates can significantly attenuate corneal epithelial cell adhesion and spreading by disruption of integrin/non-integrin receptor-matrix interactions. Descemet's membrane is susceptible to Maillard chemistry and, indeed, corneal endothelial cells show apoptotic responses after exposure to AGEs and are also known to express both RAGE and galectin-3 although the modulatory role of these receptors in corneal disease remains ill-defined.

AGEs also accumulate in the aging cornea as they do in extracellular matrix proteins in other tissues. A recent epidemiological study by Kessel and colleagues using twins has shown that glycation-related fluorophore accumulation in the cornea is linked to smoking and glucose control. Such age-related cross-linking occurs largely on the collagen component of the cornea (stroma and lamina) and can be effectively reversed using aspirin-like analgesics that have antiglycation proper-
Patients with keratoconus show enhanced CML accumulation in their corneas, while paradoxically, it has been proposed that exploitation of AGE-mediated cross-linking could have benefits for stiffening the weakened cornea of keratoconus patients.

**Lens**

When third-world demographics are taken into account, cataract formation is by far the leading cause of visual impairment across the globe. While there are many causes of lens opacity, aging is by far the major risk factor with excessive ultraviolet light exposure and associated free radical damage of crystallins being important pathogenic factors. The role of Maillard reaction in cataract formation has also been extensively studied in both the aged and diabetic lens where AGEs of various derivations and molecular structures are significantly elevated. Glycation generates age-related alterations in lens fiber membrane integrity and tertiary structure of lens proteins. This leads to aggregation and covalent cross-linking of lens crystallins that, irrespective of cataract formation, can result in reduced deformability with accompanying presbyopia. The action of dicarbonyl compounds, such as glyoxal and methylglyoxal, is enhanced in diabetes and aging, leading to AGE cross-links on α-crystallins with resultant loss of chaperone activity, increased αβ-crystallin content, and dense aggregate formation. Irrespective of the AGEs formed, it is known that diabetic patients accumulate these adducts faster than age-matched non-diabetic controls, and AGE-inhibiting compounds may have efficacy in the prevention of diabetes-related cataract formation.

Tobacco curing involves Maillard chemistry and it has been demonstrated that smoking can lead to high circulating levels of AGE-modified peptides in serum. Tobacco smoking may be a rich source of reactive glycation products, capable of promoting AGE cross-link formation in vivo. In a study of cataractous lenses there were significantly higher levels of immunoreactive AGEs in those patients with a history of smoking. Smoking remains a clear risk factor for cataract formation, and it may be concluded that tobacco smoking-related elevation in serum AGEs may act in concert with heavy metal deposition and oxidative stress to precipitate cataract formation. Indeed, metal-catalyzed Fenton reactions, which often culminate in hydroxyl radical generation, may have pathogenic significance in cataract formation, especially in diabetic patients where there is a significant accumulation of copper in the lens cells. Recent evidence suggests that a close association exists between advanced glycation, metal ions, and generation of free radicals during age-related cataract formation, where AGE-formation on crystallins leads to binding of redox active copper, which in turn catalyzes ascorbate oxidation. It has also been suggested that AGE-adducts on lens proteins may accentuate UVA-induced damage to the lens of diabetic and aged patients.

**Vitreous**

The posterior segment of the eye is filled with a clear vitreous gel that interfaces with the retina and is composed largely of a complex network of cross-linked collagen (type II, V/IX, and XI) fibrils and the hydrophilic glycosaminoglycan hyaluronan. Disorders of the vitreous gel often manifest themselves as morphological
changes to the collagen component within the cortical gel and age-related vitreous degenerations are usually a direct result of dissociation of collagen and hyaluronan. Structural changes to the vitreous, such as liquefaction and posterior vitreous detachment (PVD), are associated with aging while in diabetics such changes occur earlier than in nondiabetics in a condition sometimes called diabetic vitreopathy.

The molecular basis of vitreous degeneration remains equivocal. In terms of Maillard chemistry, it has been demonstrated that glycation can induce abnormal cross-links between vitreal collagen fibrils leading to dissociation from hyaluronan and resultant destabilization of the gel structure. Moreover, AGEs have been described in human vitreous where they correlate with age and accumulate at an even higher level in diabetic patients. The significance of this has also been shown in vitreous incubated \textit{ex vivo} in 25 mM glucose where immunoreactive AGEs formed on the vitreous collagen component and resulted in enhanced cross-linking of the fibrils—a process that could be significantly inhibited by the AGE-inhibitor amino-guanidine. Sebag has described the pathogenesis of vitreous degeneration in diabetics as a process of “precocious senescence” and while other nonglycational physiological and biochemical processes contribute to vitreous degeneration it seems likely that AGEs play a significant role in diabetic and aging vitreous dysfunction.

**AGE-RELATED DYSFUNCTION IN RETINA**

A range of age-related changes have been described in the eye. Prominent in these is the retinal pigment epithelium (RPE) and underlying Bruch’s membrane in which aging has been described through fundus analysis and histopathology. The most prominent age-related alterations include extracellular deposits of drusen, basal laminar deposits (BLDs), and changes in the chemical composition, physical structure and hydrodynamics of Bruch’s membrane. Such abnormalities are thought to be important in the development of age-related macular degeneration (AMD). Drusen and BLDs form between Bruch’s and the RPE and although the histopathologic characteristics of the deposits are well documented, their precise chemical composition has only been partly resolved. Drusen and, to a lesser extent, BLD are thought to have deleterious effects on RPE function while the accumulation of lipofuscin in RPE with age has a direct influence on outer retinal integrity. Lipofuscin accumulation in RPE may reflect accelerated phagocytosis of defective rod outer segments and/or impaired degradation of engulfed photoreceptor remnants due to altered digestibility or failure of lysosomal activity. Impaired RPE processing of shed photoreceptor outer segments is associated with drusen formation although the precise pathogenesis is poorly understood.

Association exists between Maillard chemistry and aging changes at the outer retina with reports that AGEs accumulate in drusen and in Bruch’s membrane with age and occur at a higher level in patients with AMD. Further evidence linking AGE accumulation to AMD can be surmised from the composition of drusen, which contains lipids, TIMP-3, clusterin, serum albumin, apolipoprotein E, amyloid, and vitronectin. It is significant that some of these proteins have been shown to be readily modified by AGEs and/or ALEs during aging. AGE cross-link ac-
cumulation is a feature of extracellular matrix dysfunction during diabetes, and it is significant that Bruch’s membrane is known to thicken progressively in older patients and become less permeable.\textsuperscript{71,91,92}

AGEs influence RPE \textit{in vitro} where they induce an upregulation of vascular endothelial growth factor and platelet-derived growth factor-B (PDGF-B) (VEGF).\textsuperscript{82,93,94} Prolonged exposure of RPE to AGEs or AGE-forming dicarbonyls can induce apoptotic death in these cells, often by inducing changes in intracellular pH\textsuperscript{95} with an important bearing on RPE function, maintenance of the choriocapillaris, and integrity of the RPE/photoreceptor complex. The accumulation of lipofuscin and reduction of proteolytic capacity in RPE may reflect AGE formation and receptor-mediated transport of these adducts to the lysosomal compartment. Significantly, intracellular sequestration of these highly reactive adducts can markedly reduce degradative enzymatic activity in other types of epithelial cells.\textsuperscript{96–98} Incomplete proteolysis of phagocytosed photoreceptor outer segments is linked to the formation of lipofuscin in RPE\textsuperscript{99} and it is notable that Maillard reactions appear to play an important role in the formation of age-related intracellular fluorophores and lipofuscin granules in postmitotic epithelial cells.\textsuperscript{100}

Age-related changes to retinal ganglion cells and the optic nerve head is a recognized phenomenon with an etiologic role in the pathogenesis of chronic open-angle glaucoma.\textsuperscript{101,102} It is perhaps unsurprising that products of Maillard chemistry have been detected within the collagenous matrix of the lamina cribrosa within the optic nerve head, where AGE levels correlate with age.\textsuperscript{103,104} The lamina cribrosa plays an important role in supporting the optic nerve axonal structure and the AGE-mediated cross-linking of this matrix may reduce flexibility and perhaps induce age-related axon damage characteristic of this degenerative glaucomatous disease.\textsuperscript{104} Indeed, it has been demonstrated that inhibition of AGE formation in diabetic rats effectively prevented diabetes-induced myelinated optic nerve atrophy.\textsuperscript{105}

**DIABETIC RETINOPATHY**

Retinopathy is the most common microvascular complication of diabetes and remains a prevailing cause of blindness in patients of working age in developed countries.\textsuperscript{106} With type 1 diabetes of 10-year duration, the prevalence of diabetic retinopathy is around 80\% and increases to ∼95\% by a duration of 20 years.\textsuperscript{106} The pathogenesis of diabetic retinopathy is not completely understood at a cellular and molecular level and the options for effective therapeutic intervention early in the disease process remain extremely limited. Large-scale epidemiological trials have established that hyperglycemia is an underlying cause of this disease in both type 1 and type 2.\textsuperscript{107,108} Failure to regulate blood glucose leads to biochemical abnormalities in diabetic cells and tissues and the range of pathologic lesions in retina and other vascular beds are indicative of a complex interplay between hyperglycemia-induced metabolic and hemodynamic pathways.

Short- or long-term exposure to the diabetic milieu results in a host of biochemical and metabolic abnormalities,\textsuperscript{109–111} however it remains equivocal how much each contributes to retinal pathophysiology in diabetes. Inhibition of many key pathways can show protection against multiple or specific microvascular complications in diabetic animal models, including retinopathy and it is worth stressing that acti-
vation of PKC βII, alterations in hemodynamics, oxidative stress, flux through the hexosamine pathway, and AGE formation should not necessarily be viewed as independent phenomena. Indeed, studies using the transketolase activator benfotiamine indicate that it can inhibit a common convergent pathway and effectively prevent retinopathy in diabetic animals.112

In terms of Maillard products and diabetic retinopathy, clinical studies have demonstrated that the levels of AGEs in serum,113–115 skin,116 or cornea117 correlate with the onset or grade of diabetic retinopathy. AGEs are significantly elevated in diabetic pre-pubescent children and adolescents who have background or pre-proliferative retinopathy compared to counterparts who are free from clinical signs of the disease.118 While many of the reported studies measured a range of ill-defined AGE moieties, others evaluated defined adducts such as CML, pentosidine, or crossline119,120 in association with diabetic retinopathy. At the same time, some studies have reported no correlation between AGE levels and retinopathy in diabetic patients,115,119 although the apparent disparity with other studies may be related to variations in patient populations and/or the nonuniform assays for plasma AGE quantification.

As demonstrated in other microvascular beds, AGEs and/or late Amadori products have been localized to retinal vessels and neuroglia of diabetic patients,121–126 where they would be expected to have a range of deleterious effects on cell function. In vivo and in vitro studies suggest that elevated AGE level occurring in diabetes may be an important factor in retinopathy initiation and progression. Retinal pericytes accumulate AGEs during experimental diabetes in animal models,125 which would be expected to have a detrimental influence on cell function and survival, especially since these cells have a much lower replicative capacity when compared to retinal microvascular endothelium.127 Indeed, studies have shown dysfunctional effects on retinal pericytes, such as impaired phospholipid hydrolysis and phospholipid enzyme inhibition128 or modification of the antioxidant enzymes catalase and superoxide dismutase.129 Clearly, AGEs are toxic to retinal pericytes,130–132 perhaps in an AGE-receptor mediated fashion131 and recent evidence also suggests that these adducts can induce osteoblastic differentiation and calcification in pericytes133 and a potent apoptotic death response.134 In vivo, retinal pericytes are surrounded by vascular basement membrane and lie outside the blood–retina barrier (BRB). Our own group has developed an in vitro system whereby pericytes are grown on a “diabetic-like” AGE-modified basement membrane. Exposure to this system induces pericyte dysfunction and apoptotic death, a response that can be rescued by immobilizing PDGF-BB on the matrix.135

Exposure of retinal cells in vitro and in vivo to preformed AGEs are known to cause significant upregulation of vascular endothelial growth factor (VEGF).93,134,136,137 In addition to its importance in neovascularization during proliferative diabetic retinopathy, VEGF is also a potent vasopermeability factor in the retinal microvasculature, with a role in inner blood–retina barrier (iBRB) dysfunction.138 Excessive vasopermeability is a pathophysiological hallmark of diabetic retinopathy and there is evidence to suggest that AGEs could play a role in compromise of the capillary unit leading to subtle and overt breakdown of the iBRB. Loss of iBRB integrity is observed in nondiabetic rats infused with AGE-modified proteins137 with a concomitant increase in intracellular adhesion molecule-1 (ICAM-1).139 Indeed, it is recognized that proinflammatory pathways may be active during diabetic retinopathy, manifested by increased levels of adhesion molecules
such as ICAM-1 on the surface of retinal microvascular endothelial cells. In combination with an enhanced stickiness and reduced deformability of blood-borne leukocytes in the diabetic state, this can lead to a marked leukocyte adhesion to endothelium that precipitates capillary occlusion and vascular cell death. AGEs are a possible pathogenic factor in proinflammatory responses and they can enhance ICAM-1 expression in macrovessels and have now been shown to evoke similar responses in the retinal microvascular endothelium both in vitro and in vivo.

THERAPEUTIC OPTIONS IN MAILLARD CHEMISTRY

For a range of eye diseases, inhibition of the Maillard reaction in vivo and prevention of AGE/ALE-mediated cell toxicity has exciting possibilities. There have been many approaches to either prevent AGE-formation, reduce AGE receptor-ligand interactions/signaling pathways effects, or break established AGE cross-links. These treatments not only offer an important insight into the pathogenic role of AGEs in diabetic retinopathy but may have clear applicability to the treatment of patients with other ocular diseases.

Amadori product formation is an important basis of Maillard chemistry in biological systems because progression to cross-link pathology requires chemical rearrangement to create reactive intermediates before the formation of irreversible AGEs. An important pharmacological strategy for the inhibition of this process has utilized the small nucleophilic hydrazine compound aminoguanidine (Pimagedine). This drug is a potent inhibitor of AGE-mediated cross-linking and has been shown to prevent a range of diabetic vascular complications in experimental animals (reviewed by Vasan and colleagues), including diabetic retinopathy. Aminoguanidine has been evaluated in a multicenter clinical trial where it failed to achieve statistically significant lowering of serum creatinine and urinary albumin, but showed positive signs towards slowing the progression of overt nephropathy and retinopathy progression.

Another anti-AGE strategy is to attack AGE cross-links formed in biological systems. This constitutes an exciting approach since it would “break” preaccumulated AGEs and subsequently allow their clearance via the kidney. An AGE cross-link “breaker” prototype has been described to attack dicarbonyl-derived cross-links in vitro and there are now at least two such (related) chemical agents with the ability to reduce tissue AGEs in experimental diabetes. The “breaker” ALT-711, has been shown to ameliorate myocardial stiffness in aged dogs and improved the ability of the carotid artery to expand during systole in diabetic rats. In preliminary clinical trials, ALT-711 modestly improved arterial compliance in aged patients with measurable cardiovascular stiffening. The effects of ALT-711 on retinopathy have yet to be evaluated.

Another successful approach has been to screen for compounds with post-Amadori product scavenging potential, since this is an important route for AGE formation in vivo. So-called Amadorins have an ability to scavenge reactive carbonyls and therefore inhibit the conversion of Amadori intermediates to AGEs and also ALEs. Aminoguanidine possesses no scavenging properties, but it has been found that the derivative of vitamin B6, pyridoxamine (Pyridorin™) is an efficacious and specific post-Amadori inhibitor, with the ability to prevent re-
nal dysfunction in diabetic rats. Also in diabetic rats, pyridoxamine successfully reduced retinal AGE accumulation and also preventing upregulation of basement membrane–associated genes and diabetes-associated capillary acellularity.

**CONCLUSION**

The Maillard reaction may play a pathogenic role in diabetes and age-related dysfunction of the eye. The pathogenesis of such disorders are multifactorial and it is clear that advanced glycation, whilst playing a significant role, is not the only process leading to cell and tissue dysfunction. Nevertheless, important events in diabetes and aging, such as free radical generation, may have important links to or are secondary consequences of Maillard chemistry. The accumulation of AGEs may represent a function of the aging process, but their pathogenic role in the eye still needs to be directly and unequivocally proven. Whatever their place in the pathogenic hierarchy of ocular disease, products of the Maillard reaction may play a significant role in eye diseases and, as research continues, novel pharmacologic intervention strategies may alleviate some of the sight-threatening complications suffered during diabetes and aging.

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