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Chapter

Advanced Glycation End Products: Formation, Role in Diabetic Complications, and Potential in Clinical Applications

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Abstract

Hyperglycemic conditions and disruptions to glucose-regulating pathways lead to increased formation of highly reactive aldehydes, methylglyoxal and glyoxal, which react with certain arginine and lysine residues in proteins to form advanced glycation end products (AGEs). These AGEs damage the integrity of the retinal vasculature predominantly through two mechanisms: non-receptor-mediated damage, which pertains to the interaction with extracellular matrix and its functional properties, and receptor-mediated damage through AGE interactions with their receptors (RAGE) on pericytes and Muller cells. Damage occurring between AGE and RAGE potentially generates reactive oxygen species, inflammatory cytokines, and growth factors. Both mechanisms result in increased permeability of endothelial tight junctions, and this increased permeability can lead to leaking and eventually ischemia. Once this ischemia becomes significant, neovascularization can occur, the hallmark of proliferative diabetic retinopathy. Current pharmaceutical studies have shown the potential of AGE inhibitors, such as aminoguanidine, in decreasing AGE production, thus minimizing its effects in hyperglycemic conditions. Other pharmaceutical interventions, such as Tanshinone IIA, aim to protect cells from the impacts of AGEs. Future research will not only continue to understand the properties of AGEs and their effects on diabetes and diabetic complications like diabetic retinopathy but will also explore how they impact other diseases.

Keywords: advanced glycation end products, oxoaldehyde, RAGE, hyperglycemia, diabetic retinopathy, inflammation, cytokine, neovascularization

1. Introduction

Advanced glycation end products (AGEs) are formed through a non-enzymatic process in hyperglycemic conditions, and they impact the retinal vasculature negatively through the formation of reactive oxygen species, secretion of aberrant proteins or growth factors, alteration of the extracellular matrix, and secretion of inflammatory cytokines [1]. It is important to consider the difficulty of differentiating the effects of hyperglycemia from those of AGEs, as AGE concentration is controlled by glucose levels. Because of this, occasionally high glucose levels are

measured interchangeably with high levels of AGEs. There are two primary mechanisms by which AGEs damage the retinal vasculature which will be discussed in this chapter: interactions with RAGE (AGE receptors) and damage to the extracellular matrix [2]. While these two mechanisms work differently, both pathways result in thickening of the basement membrane which impairs signaling between cells of the microvasculature hindering their structure and increasing rigidity, which leads to the hemorrhagic signs seen in patients with diabetic retinopathy (DR) [3]. Endogenous anti-stressors are important for the management of high levels of AGEs through various mechanisms, but many times are not sufficient to control the progression of DR [2]. Thus, it is important to modify the production of AGEs through exogenous mechanisms, such as nutrition, reducing smoking, or treating the condition through medication [2].

2. AGE formation

Advanced glycation end products (AGEs) were first discovered in the early 1900s by the Maillard reaction process. Scientists discovered that when amino acids were heated in a mixture with reducing sugars, the reaction turned a yellowish brown color. Further studies indicated that reducing sugars, i.e., glucose, reacted non-enzymatically with the amino acid reagents to form Schiff bases, an early glycation product, and Amadori products, intermediate glycation products. AGE formation can utilize other reagents such as lipids, connective tissue extracellular matrix, and nucleic acids. The process of glycation is enhanced by diabetic complications and occurs in the earlier stages of the Maillard reaction; intracellular sugars, such as glycolytic pathway intermediate glucose-6-phosphate, are glycated at a faster rate than glucose. Amadori products are α-dicarbonyls (oxoaldehydes) such as 3-deoxyglucosone (3-DG) and methylglyoxal (MGO) which is formed by the non-oxidative rearrangement of Amadori adducts from fructose-3-phosphate in the polyol pathway. This pathway has also been studied as a precursor to hyperglycemia-induced damage in diabetes. Methylglyoxal and 3-deoxyglucosone are formed in the early stages of glycation processes: degradation of glucose, Schiff's bases, and from Amadori products; these oxoaldehyde products can serve as a checkpoint in the AGE pathway since an accumulation of these products is an implication of accelerated vascular damage [4, 5].

3. Mechanism of action

The main mechanisms of AGE that affect cells are the adducts on proteins (including N-carboxymethyllysine, pentosidine, or hydroimidazolone) that can interact via AGE ligand-gated receptors such as RAGE on endothelium that lead to secretion of cytokines TNF-α and VEGF; AGEs can stem from exogenous and endogenous adducts due to glucose metabolism. RAGE is the most widely studied AGE receptor found on endothelial cells in vasculature and on macrophages and microglia. AGE interacts with RAGE on macrophages, leading to intracellular generation of free radicals and oxidative stress, which are then phosphorylated by MAP kinase to activate NF-κB and increase expression of NF-κB controlled genes to cause vasoconstriction, enhanced adhesion molecule expression, and induce a procoagulant state. An overexpression of RAGE leads to oxidative stress and NF-κB activation. Current studies show that cross-linked AGEs with RAGE on proteins are closely linked with diabetic retinopathy progression. In the diabetic retina, AGE and adducts are found on vascular cells, neurons, glia, and in elevated levels in Muller macroglia—these specialized retinal cells show

increased dysfunction in hyperglycemic and hypoxic conditions that lead to more AGE formation. AGEs induce oxidative stress and consequent apoptosis of retinal pericytes; furthermore, AGEs induce the closure of intercellular junctions between endothelial cells [4–7].

3.1 AGE-RAGE interactions

Inflammation is an important component in the progression of diabetic retinopathy (DR), and AGEs induce this process through interaction with receptors on the cell surface called RAGEs. These receptors are found on most cells, meaning that AGEs exert a wide effect on many different organs. In DR, results of AGE-RAGE interaction on inflammatory cells such as macrophages and lymphocytes, and on microvascular cells such as endothelial cells or pericytes are thought to produce a significant impact on the progression of DR [8]. Monocytes and lymphocytes secrete inflammatory cytokines through the induction of NF-κB [9], production of IL-1, IL-6, IL-8, MCP-1 and TNF-α, and upregulation of adhesion molecules such as VCAM and ICAM [9]. IL-8 and TNF-α levels are elevated in patients with nonproliferative diabetic retinopathy (NPDR), signifying the increased inflammation in the early stages of DR. These cytokines are produced by activated neuronal cells and endothelial cells, and they exert their effect by causing early neuronal cell death in the retina [9]. Inflammation negatively impacts the retinal vasculature by altering the action of vascular cells which leads to the upregulation of various proteins that contribute to the thickening of the basement membrane. MIP-1, IL-3, and IL-1 are thought to play a role in angiogenesis [9, 10], which would facilitate the progression from NPDR to proliferative diabetic retinopathy (PDR). Communication between glial cells and neurons is imperative for maintenance of the vasculature, and it has been shown that inflammation can impede the crosstalk between these cells early in the disease process [9]. Thickening of the basement membrane is one of the leading mechanisms by which crosstalk amongst cells of the retinal vasculature are impeded. This crosstalk is essential for many processes such as providing energy to retinal vascular cells and maintaining homeostasis [9]. In endothelial cells, AGE-RAGE interaction has been shown to increase proliferation via increased VEGF production induced through the MAPK pathway [10, 11]. This process contributes to angiogenesis and accelerates the progression of DR from NPDR to PDR. In pericytes, an opposite effect has been observed, as increased AGE-RAGE interaction leads to apoptosis of these cells, which is one of the first steps in the pathogenesis of DR [11]. As pericyte dropout occurs, the vasculature becomes less regulated leading to hemorrhage and leaking.

3.2 Oxidative stress

Reactive oxygen species (ROS) accumulate in DR from the conversion of glucose to fructose through the NADPH pathway. This accumulation of ROS leads to increased production of AGEs, which then exert their effects through AGE-RAGE interactions or by crosslinking extracellular matrix proteins. One of the outcomes of AGE-RAGE interactions is production of ROS as well, leading to enhanced concentrations of ROS and further progression of the disease. Aldose reductase, which is upregulated to compensate for the high levels of glucose and is essential for the conversion of glucose to fructose, activates a serine/threonine-related protein kinase PKC-δ. Protein kinase PKC-δ is known to inhibit platelet derived growth factor survival activity, an essential pathway for pericyte proliferation and survival. Considering that pericyte loss is typically the initial step in the pathogenesis of DR, this explains the role of ROS in the early stages of DR [12].

3.3 Impact on the extracellular matrix

The other predominant mechanism of damage from AGEs pertains to their effect on the extracellular matrix of the retinal basement membrane. Inflammation induced by AGEs that was discussed above has a significant impact on the basement membrane, specifically from the elevated levels of inflammatory cytokines IL-1β and TNF-α which induce the production of extracellular matrix proteins. As these excess proteins accumulate in the extracellular matrix, the basement membrane begins to thicken. When AGEs attach to collagen or elastin in the extracellular matrix, it causes the collagen to be less susceptible to hydrolytic breakdown and becomes less flexible. It has also been found that glycation increases the production of collagen and other extracellular matrix proteins, along with the increase in production induced by inflammatory cytokines. This increased production and crosslinking of collagen along with the decreased elastin levels significantly increase the rigidity of the microvasculature through stiffening and thickening of the basement membrane [2, 10, 13].

The accumulation and crosslinking of extracellular matrix proteins contributes to the thickening of the basement membrane, which hinders its integrity ultimately leading to the hemorrhagic pathologies that occur as a result of diabetic retinopathy. The initial damage caused by this thickening is decreased perfusion of the retinal capillaries, leading to occlusion or degeneration of these capillaries [14]. This is one of the characteristic steps in NPDR: ischemia caused by lack of oxygen perfusion sets off the cascade of events that leads to neovascularization, the hallmark of PDR. When looking at the sequelae following the impact of AGEs on the basement membrane, it suggests that AGEs play a significant role in the progression and pathogenesis of diabetic retinopathy. A study showed rats with diabetes tested positive for AGEs (periodic acid/Schiff reagent positive material) at significantly higher levels than those under normal conditions [15]. Rats with diabetes also demonstrated a twofold increase in acellular retinal capillaries over the course of 26 weeks compared to their wild type counterparts, and diabetic rats also experienced significant capillary closure over the course of 75 weeks.

4. Dietary and exogenous sources

Processing of foods at high temperatures using the Maillard reaction to enhance flavoring and color subsequently leads to the formation of reactive aldehydes that leads to formation of advanced glycation end products, which are also formed naturally in body tissues. Studies depicted that canned meats, nuts, and grain-based products contained the highest levels of AGE, and coffee, butter, vegetables, and fruits as well as food prepared by steaming or boiling contained the lowest amounts of AGE [5, 16].

Research studies show that the average amount of AGE consumed on a daily basis by an individual range from 12,000 to 20,000 kilo-units (kU) of AGEs/day with diabetic subjects consuming a range of 4000–24,000 kU AGEs/day. Pyrraline is one of the most common AGE adducts and may be found in milk and bread crust, while pentosidine, another AGE adduct, is found in pretzel sticks and in its free form in coffee. Study of individual AGEs suggest that protein-bound pentosidine is not as readily absorbed as free pentosidine, therefore, increased levels free AGE in urine and plasma is correlated to AGE-rich dietary intake. Intake of elevated levels of sodium, carbohydrates, and vitamins were found to not be associated with DR risk or progression. Relationship between dietary AGE and promotion of AGE formation in the body tissues will require new research since current research has only centered on skin autofluorescence before and after intake of AGE-rich foods [5, 16].

The effects of dietary AGE were examined in several studies. AGE-poor diets depicted improved biomarkers for oxidative stress, endothelial, and inflammation in healthy subjects, and restricted AGE diets showed decreased levels of oxidative stress in diabetic patients as well as decreased insulin resistance and reduced levels of low-density-lipoprotein. Other studies have also found that dietary AGEs affect inflammatory markers including cytokine TNF-α, and AGE-poor diets have led to decreased risk for cardiovascular disease and endothelial dysfunction. Several studies have also examined the effects of dietary AGE on motor functions, finding that increases in oxidative stress and inflammation due to high levels of AGE lead to muscle stiffness and loss of elasticity [5, 16].

5. Physiological alterations due to hyperglycemic conditions

Hyperglycemic conditions initiate formation of AGE and promote biochemical abnormalities that involve formation of AGE. The three main AGE formation biochemical abnormalities include flux via hexosamine pathway, diacylglycerol-mediated activation of PKC-β with benfotiamine, and the stimulation of transketolase activity that induces excess triose phosphates to undergo the pentose phosphate pathway [17, 18].

The primary precursor of AGE is glucose, but other carbonyl precursors exists, though diminutively less reactive, including glyoxal, methylglyoxal, and 3-deoxyglucosone that result from glycolysis. The levels of AGE in the body tissues increase significantly in complications of disease such as diabetic retinopathy, but it is the accumulation of AGE that results in accelerated complications of diseases. Body cells have innate detoxification systems that prevent accumulation of AGE precursors such as methylglyoxal, and detoxification properties of enzymes may be essential in further research about prevention of diabetic retinopathy complications. Deterioration of kidney function leads to accumulation of AGEs, thus leading to endothelial abnormality and vascular disease [4, 5, 17].

6. Treatments

No cure for diabetic retinopathy has been discovered yet, despite many efforts from various clinical trials. The standard pharmacological treatment currently for diabetic retinopathy is anti-VEGF injections, which aids in the stabilization and halts progression of the disease [19]. This approach has only been successful in treating about two-thirds of the population and the best second-line pharmacological therapy has not been identified [19]. These factors have spurred the search for a better alternative, especially agents which combat the AGEs and their effects directly. There are different categories of treatments against AGEs, but the most widely studied treatments include those that specifically inhibit AGEs themselves as well as lifestyle changes to reduce the production of AGEs.

6.1 Direct AGE inhibitors

The first direct AGE inhibitor that garners the most promise is aminoguanidine, which inhibits AGE formation on both collagen and the basement membrane [15]. As discussed above in the section about AGE's impact on the extracellular matrix, AGEs crosslink collagen and other proteins in the basement membrane and extracellular matrix which causes it to thicken, lose its integrity and ultimately become leaky. By inhibiting the formation of these crosslinked proteins, the basement membrane and extracellular matrix can preserve their integrity and the normal communication

between pericytes and endothelial cells can continue. A study demonstrated that rats treated with aminoguanidine showed significantly less AGE deposition in the basement membrane/extracellular matrix and overall healthier capillaries [15]. Treatment with aminoguanidine also reduced endothelial cell proliferation in diabetic retina, which is another pathological change associated with diabetes. The downside is that this treatment was unable to completely resolve all of the pathological processes of diabetes, namely the occurrence of retinal microaneurysms. In untreated diabetic rats, 38% demonstrated microaneurysms while those treated with aminoguanidine reduced the incidence to 20% (0% in controls). This improvement is promising, but microaneurysms lead to vessel destruction, which advances the progression of NPDR to PDR, the more detrimental stage of DR. An alternative study demonstrated an even greater decrease in microaneurysms, but their sample size was small (a single retina) and it was conducted in dogs rather than rats [20].

Another direct AGE inhibitor is pyridoxamine. This compound has been found to decrease glycation of proteins in the extracellular matrix as well as decrease the formation and production of AGEs. A study measured the success of treating diabetic retinopathy with pyridoxamine by the quantity of acellular capillaries formed over a period of time [4]. Acellular capillaries are nonperfused capillaries which result from a variety of factors onset by diabetes such as pericyte dropout, extracellular matrix, and endothelial damage [21]. After 29 weeks, it was found that diabetic rats treated with pyridoxamine showed similar amounts of acellular capillaries to controls. It also demonstrated the impact of pyridoxamine on the production of extracellular matrix proteins, which are upregulated in diabetic retinopathy. Pyridoxamine significantly reduced the production of extracellular matrix proteins like collagen type IV and laminin, close to the levels found in controls [4].

6.2 Other pharmaceutical interventions

Besides the usage of direct AGE inhibitors, other drugs options are being explored. One such drug is Tanshinone IIA (Tan IIA). Tan IIA is derived from the roots of *Salvia miltiorrhiza*, which is a plant that is used in traditional Chinese medicine. Studies indicate that Tan IIA impacts several of the negative effects that hyperglycemic conditions have on human retinal endothelial cells. Tan IIA has an inhibitory effect on proliferation, migration, and vascularization in human endothelial cells and has some correlation to VEGF expression [22]. In terms of AGEs, a recent study explored how Tan IIA protects retinal endothelial cells from the impacts of AGEs, specifically cell dysfunction resulting from the presence of MGO. The study showed that MGO impacted cell viability negatively in a dose-dependent manner. Treatment of the cells with Tan IIA increased their viability in conditions where MGO was also present. MGO presence also resulted in mitochondrial fission in bovine retinal endothelial cells, and the presence of Tan IIA protected against this type of AGE-induced injury in the cells [23].

6.3 Lifestyle modification

Exogenous AGEs are AGEs that are consumed and produced through diet and lifestyle, and they differ from the endogenous AGEs that form in hyperglycemic conditions metabolically. Because of this, diet and lifestyle changes are arguably the most important treatment in DR, as diet is a significant contributor to exogenous AGEs found in the form of foods high in protein and fat. Pertaining to lifestyle, smoking tobacco products is associated with higher levels of AGEs in serum which contributes to the progression and risk of DR [24]. Overall, a decreased calorie intake, a modified diet, and smoking cessation have been shown

to increase risk and overall disease progression of DR and should be an important treatment regimen, in addition to pharmacological treatments with AGE inhibitors, in all patients with DR.

7. Conclusion

Advanced glycation end products (AGEs) are formed in increasing amounts due to hyperglycemic conditions implicated in diseases such as diabetic retinopathy. Endogenous AGEs are products from metabolic pathways that follow the Maillard reaction with the oxidation of oxoaldehydes. Exogenous AGEs may come from food sources processed at high temperatures, which increased the amount of reactive aldehydes in the food. Several studies have indicated that inhibition of AGEs holds high potential in the treatment of diabetic retinopathy. Aminoguanidine, a nonspecific inhibitor of AGE, holds the most pharmaceutical promise according to several studies conducted, but other drugs such as Tanshinone IIA are also promising. However, alterations of lifestyles may also provide highly favorable results in decreasing the amount of AGE produced and consumed by the body.

Diabetes and the complication of diabetic retinopathy are gradually on the rise and are widespread. Another disease that is nearly as widespread and also equally relevant in scientific study is Alzheimer's disease. Recent studies indicate that there may be a link between the two and the factors that are known to impact one of those diseases, such as AGEs, also have effects on the other [25]. Of organs that may exhibit diabetic complications, the eye and its associated connections are one that are closest physically to the brain, and one long-term study on retinal health and cognitive dysfunction showed that 40% of patients with DR showed reduced cognition [26]. Future studies in relation to AGEs will not only focus on the properties of AGEs and their impact on diabetes and its complications, but also how other illnesses are impacted by them as well.

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