Advanced glycation end products and diabetic retinopathy

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Abstract Studies have established hyperglycemia as the most important factor in the progress of vascular complications. Formation of advanced glycation end products (AGEs) correlates with glycemic control. The AGE hypothesis proposes that hyperglycemia contributes to the pathogenesis of diabetic complications including retinopathy. However, their role in diabetic retinopathy remains largely unknown. This review discusses the chemistry of AGEs formation and their patho-biochemistry particularly in relation to diabetic retinopathy. AGEs exert deleterious effects by acting directly to induce cross-linking of long-lived proteins to promote vascular stiffness, altering vascular structure and function and interacting with receptor for AGE, to induce intracellular signaling leading to enhanced oxidative stress and elaboration of key proinflammatory and prosclerotic cytokines. Novel anti-AGE strategies are being developed hoping that in next few years, some of these promising therapies will be successfully evaluated in clinical context aiming to reduce the major economical and medical burden caused by diabetic retinopathy.

Keywords Advanced glycation end products (AGEs) · Hyperglycemia · Diabetes mellitus · Microvascular disease · Retinopathy

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Introduction

Diabetes is a common condition with multiple complications [1]. Diabetes mellitus is increasing at an alarming rate, particularly the non-insulin-dependent diabetes mellitus (NIDDM) [2]. Diabetic complications are a major cause of morbidity and mortality and are an ever-increasing burden to healthcare authorities all over the world [3].

Hyperglycemia is mostly considered to be the major cause of diabetic microvascular complications and epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progress of diabetic complications [3]. But the exact mechanism of the deleterious effect of hyperglycemia on the small and large blood vessels is not known [4]. Some prospective clinical studies have shown a strong relationship between glycemia and diabetic macro- and microvascular complications in both types 1 and 2 diabetes [3, 5, 6].

Mean increase of blood glucose concentrations in patients with diabetes mellitus and the development of degenerative microvascular changes have been found co-related [7, 8]. One mechanism linking chronic hyperglycemia with tissue damage such as that in diabetic retinopathy is the formation and accumulation of advanced glycation end products (AGEs) [9]. Thus, advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) might be the contributors to accelerated micro- and macrovasculopathy observed in diabetes [6]. Hence, the study of AGE represents one of the most promising areas of research today [10].

Formation and structure of advanced glycation end products

The nonenzymatic reaction of sugars with proteins through the Maillard reaction after undergoing multiple steps finally leads to the formation of AGEs [8]. 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 [11–15]. If all or even one 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 [12, 16]. This undergoes Amadori reorganization where these highly reactive intermediate carbonyl groups, known as α -dicarbonyls or oxoaldehydes accumulate [17, 18]. This buildup is termed as “carbonyl stress.” The α -dicarbonyls have the ability to react with amino, sulfhydryl, and guanidine functional groups in proteins [19]. The reaction results in denaturation, browning, and cross-linking of the targeted proteins [19, 20]. Also 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 [21]. AGEs once formed are almost irreversible [13].

AGEs are large heterogeneous group and total number of existing AGEs is not known [22]. Till now, only a small number of AGEs occurring in vivo have been completely characterized and structurally defined [23]. The major AGEs in vivo appear to be formed from highly reactive intermediate carbonyl groups, known as dicarbonyls or oxoaldehydes, including 3-deoxyglucosone, glyoxal, and methylglyoxal [24, 25]. Among the most common chemically characterized AGEs in humans include pentosidine and CML. AGEs like pentosidine have intrinsic fluorescence, and thus, tissue and plasma fluorescence can be used as markers of AGE accumulation whereas AGEs such as CML are nonfluorescent and may be detected by procedures like enzyme-linked immunosorbent assays (ELISA) [26–29].

Exogenous sources of advanced glycation end products

Along with endogenous formation of AGEs, they can also originate from exogenous sources such as tobacco, smoke, and diet [26–29]. Smokers and patients on high-AGE diet were detected to have higher serum and tissue AGE levels [26–28]. Food processing, including prolonged heat processing induce accelerated generation of glyco-oxidation and lipo-oxidation products leading to increased proportion of ingested AGEs absorbed with food [26–29]. A thermally processed conventional Western diet contains high levels of fat and sugar and can typically lead to intake of harmful adducts as high as ~25 to 75 mg AGEs/ALEs per day [30].

Effects of advanced glycation end products

AGE effects include production of reactive oxygen species (ROS), binding to specific cell surface receptors and formation cross-links [11, 31] and contribution to the pathophysiology of vascular disease in diabetes [12, 32–34]. AGEs induce irreversible crosslinks in long-living extracellular matrix [35–39] and upon binding to specific cellular proteins change the local concentrations of cytokines, growth factors, and other bioactive molecules [40–42]. AGEs accumulate in the vessel wall which might disturb the cell structure and function. AGEs have been implicated in both the microvascular and macrovascular complications of diabetes such as retinopathy, nephropathy, neuropathy, and also macrovascular disease atherosclerosis [38, 43, 44].

Morphological and functional changes of diabetic retinopathy include basement membrane thickening, loss of pericytes, and increased permeability [45]. Accumulation of AGE might contribute to increased retinal endothelial cell permeability resulting in vascular leakage [46]. Vessel wall thickening and coagulation, leading to occlusion and ischemia [47] along with induction of growth factors such as vascular endothelial growth factor (VEGF), result in angiogenesis and neovascularization [48].

Significant role of advanced glycation end products in diabetic retinopathy

A large number of studies have clearly indicated significant role of AGEs in diabetic nephropathy [8, 35, 49–53] and atherosclerotic vasculopathy [8, 35, 49, 52, 54, 55], but whether AGEs are a factor in the pathogenesis of diabetic retinopathy still needs to be explored. The role of AGE in the development of retinopathy has been suspected for years [56].

AGEs accumulate with age and at an accelerated rate in diabetes [49, 57, 58] within the various organs that are damaged in diabetes, and the accumulation rate of these AGEs is accelerated by hyperglycemia [59]. AGEs accumulate in most sites of diabetes complications, including the kidney, retina, and atherosclerotic plaques [60–62]. Studies have implicated that AGEs have been localized to retinal blood vessels in patients with type 2 diabetes and were found to correlate with the degree and clinical progression of retinopathy [61, 62]. AGEs have been quantified in various ocular tissues and were found elevated in diabetic subjects when compared to non-diabetic control subjects [63–65]. This includes vitreous collagen [66], where the AGE levels correlate with diabetic retinopathy [67]. In the diabetic retina, AGEs accumulation has been observed in vascular cells, neurons, and glia [59, 64, 68–70] which may have pathogenic implications in the individual cells and retinal function.

AGEs accumulate in retinal pericytes during diabetes [47] influencing pericyte survival and function finally leading to pericyte loss. Along with loss of pericytes, other characteristic changes including thickening of the basement membrane, hyperpermeability, and microaneurysm formation are observed [71]. Pericytes play an important role in the maintenance of microvascular homeostasis and thus loss of pericytes could predispose the vessels to angiogenesis, thrombogenesis, and endothelial cell (EC) injury, thus leading to full-blown clinical expression of diabetic retinopathy [72]. Studies have also established that the AGE–RAGE interaction elicits ROS generation in cultured retinal pericytes inducing apoptotic cell death of pericytes. AGEs induced nuclear factor- κ B (NF- κ B) activation and decreased the ratio of Bcl-2/Bax increasing the activity of caspase-3, an enzyme responsible in the execution of apoptosis of pericytes [73, 74]. AGEs also upregulate RAGE mRNA levels in pericytes through the intracellular ROS generation [75]. These positive feedback loops transduced the AGE signals, again increasing the cytotoxic effects of AGEs on retinal pericytes causing pericyte dysfunction [76].

AGEs act on pericytes to stimulate VEGF expression [74]. VEGF, a specific mitogen to ECs, also known as vascular permeability factor, is considered as a crux factor in the pathogenesis of diabetic retinopathy [77]. VEGF level was also found co-related with the breakdown of the blood–retinal barrier, thus being involved in retinal vascular hyperpermeability in background retinopathy [78, 79]. These findings suggest the involvement of AGEs in the development and progression of diabetic retinopathy by inducing VEGF over expression in pericytes. Murata et al. [64] found association between the accumulation of CML in the human diabetic retina with proliferative and non-proliferative changes and the expression of VEGF. Furthermore, AGEs interact with RAGE and directly stimulate growth and tube formation of microvascular ECs, the key steps of angiogenesis [48, 80]. It was also observed in some studies that the angiogenic activity of AGEs was mainly mediated by autocrine VEGF production by ECs. Studies have also shown that the AGE–RAGE interaction might increase VEGF gene transcription in microvascular ECs by NADPH oxidase-mediated ROS generation and subsequent NF- κ B activation via Ras-MAPK pathway [80, 81].

The role of inflammatory reaction in diabetic retinopathy has also aroused interest [82]. AGEs have been also involved in the process of vascular inflammation as AGEs were found to increase leukocyte adhesion to cultured retinal microvascular ECs by inducing intracellular cell adhesion molecule-1 (ICAM-1) expression [83]. Similar phenomenon was also seen in non-diabetic mice infused with preformed AGEs, which resulted in significant leukostasis and blood–retinal barrier dysfunction in these mice. Retinal VEGF was found to induce ICAM-1

expression leading to leukostasis and breakdown of blood–retinal barrier [84]. It was also observed that AGEs increase monocyte chemoattractant protein-1 (MCP-1) and ICAM-1 expression in microvascular ECs through intracellular ROS generation inducing T-cell adhesion to ECs [85, 86]. As the levels of MCP-1 in vitreous fluids are associated with the severity of proliferative diabetic retinopathy [87], AGEs might be one of the most important proinflammatory factors for the progression of diabetic retinopathy.

Hammes et al. [70, 88] have studied the role of AGE in the development of diabetic retinopathy and the effect of the AGE-formation inhibitor, aminoguanidine, in animal models. Rats with streptozotocin-induced diabetes developed a characteristic diabetic retinopathy with endothelial cell proliferation, pericyte loss, microaneurysms, and capillary closure. After 2 weeks of diabetes induction, aminoguanidine treatment was started which resulted in a dramatic reduction in the development of retinal lesions, 80 % reduction in pericyte loss, absence of microaneurysms and endothelial cell proliferation and prevention of accumulation of AGE at the branching sites of precapillary arterioles [88]. Aminoguanidine has prevented the development of retinopathy in the diabetic spontaneously hypertensive rat [70]. Here, the AGE-formation inhibitor completely prevented the deposition of periodic acid Schiff-positive material in arterioles and formation of microthrombi. Aminoguanidine is also proved effective in the secondary prevention of diabetic retinopathy in rats, with treatment starting 6 months after diabetes induction by streptozotocin [89]. These results are in line with those of the Diabetic Control and Complications Trial (DCCT), which indicated the efficacy of controlling blood glucose levels in primary and secondary prevention [90]. The toxic effects of high glucose concentrations on capillary pericytes have been found to be inhibited by aminoguanidine. Recent studies have also indicated that the toxic effects of AGE on retinal capillary pericytes and endothelial cells in culture can be blocked by RAGE antibodies [91]. Hammes et al. studied in diabetic rats that a 75 % decrease in basement membrane thickening in the retina of animals treated with aminoguanidine compared to non-treated animals suggested a role for AGE accumulation. This increase is markedly reduced by aminoguanidine, indicating a the role of AGE [70].

AGEs along with decrease in endothelial nitric oxide synthase mRNA levels in ECs also reduce NO (nitric oxide) bioavailability by inactivating NO to form peroxynitrite via ROS generation. Reduced synthesis and/or bioavailability of NO may accelerate vascular injury in diabetic retinopathy because NO is known to exert anti-inflammatory properties in vivo [92]. An increase in skin concentration of pentosidine in diabetic patients was found correlated with the development of proliferative retinopathy [56]. Similarly

FFI, CML, and total fluorescence increase along with the increasing severity of retinopathy [43].

Measurement of advanced glycation end products in diabetic retinopathy

Serum AGEs measured by ELISA were found co-related with diabetic retinopathy in NIDDM patients without renal dysfunction [93], though some studies have reported no correlation between AGE levels and retinopathy in diabetic patients [94]. This is so because AGE-modified proteins are spontaneously cleared from the blood, except during renal dysfunction, and thus quantification of AGEs in serum may not always prove to be significant biomarkers of the disease [95]. Contrary to that, the DCCT skin collagen ancillary study group [96] showed that AGE modification of extracellular matrix isolated from skin biopsies often provides more relevant data [39]. They found that cross-linked AGEs on long-lived skin proteins are significantly associated with the progression of diabetic retinopathy. Plasma AGE assays have not yet been shown to be directly related to tissue AGE content but as blood is more accessible for repeated measurements of AGEs than tissue requiring biopsies, it is being used for AGE quantification [97]. Due to this reason, it remains ambiguous as to which circulating AGEs should be quantified and particularly holds best biological relevance [98].

Most serum AGEs research have been performed in diabetic animals as compared to human diabetes. This is because there is no consequently recognized standard for the measurement of serum AGEs in human beings. Since different groups cannot be measured in the same way, serum levels of AGEs are measured spectrofluorometrically and by sensitive HPLC, LC/MS, or GC/MS methods [99]. Chemically defined s-AGEs, such as carboxymethylsine and pentosidine, can be measured by ELISA [100, 101]. The competitive ELISA method is mostly used for the measurement of AGEs concentrations in serum. The reaction principle is as follows: the immunoplate wells are overcoated by AGE antigen, and the serum containing an unknown quantity of AGE antigen is incubated together with an anti-AGE antiserum. At the end of the incubation period, the wells are treated with labeled secondary antibody enzyme. After that, a substrate is added, which gives the absorbance difference which is then measured. Competitive immunoreactivity of the samples is read from the calibration curve [102].

Treatment targeting advanced glycation end products

Endogenous AGEs Therapeutic interventions for reducing AGE formation include reduction in AGE formation by

reducing crosslink formation [103], by reducing AGE deposition using crosslink breakers, and by enhancing cellular uptake and degradation. Also receptor inhibition of AGE using neutralizing antibodies or suppression of post-receptor signaling, using antioxidants can be other novel strategies for targeting reduction of AGEs [104].

Exogenous AGEs Other than reduction or impairment of AGE formation and drug therapy, modifying the intake of food- and tobacco-derived AGE can be other treatment modalities to reduce AGEs. AGEs can be absorbed through the diet [105]. Foods high in protein and fat, such as meat, cheese, and egg yolk, are rich in AGEs [29]. Foods high in carbohydrates have the lowest amount of AGEs. High cooking temperatures, cooking methods like broiling and frying, and increased cooking time cause increased AGEs formation [106]. A diet loaded with AGEs result in proportional elevations in serum AGE levels patients with diabetes [105]. On the contrary, dietary AGE restriction causes a 30 to 40 % decrease of serum AGE levels in healthy subjects [106]. Patients with diabetes and renal failure who restricted dietary AGE intake demonstrated suppressed AGE-related tissue injury [29, 107]. Thus, restriction of dietary AGEs may be an effective strategy for the reduction of AGEs [108]. A low-AGE diet administered for 6 weeks in a clinical trial resulted in lower serum AGE levels and inflammatory markers such as C-reactive protein [29].

Conclusion

The onset of diabetic retinopathy is multifactorial, and a cascade of hyperglycemia-linked pathways has been involved in the initiation and progression of this disease. All cells in the retina are affected by the diabetic milieu, and not any single process is solely responsible for deranged retinal physiology. The biochemical process of advanced glycation appears to be enhanced in the diabetic milieu due to hyperglycemia along with oxidative stress and lipids. A heterogeneous group of AGEs are generated that appears to induce directly and indirectly various pathological processes ultimately enhancing the development and progression of diabetic vascular complications. The preferential appearance of intracellular AGE deposits within those cells that are most susceptible to toxicity in diabetic retinopathy, together with the simultaneous occurrence of AGE deposits and retinal pathology, are indeed intriguing. Further research is required for gaining a clear understanding of the cellular and molecular processes and to firmly establish whether AGEs are direct contributors in the initiation and progression of diabetic retinopathy.

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