Advanced glycation end products and diabetic complications: A General overview

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ABSTRACT

Diabetes mellitus, especially type 2 diabetes is increasing at an alarming rate reaching epidemic proportions. Although hyperglycemia has been considered as playing an important role in the pathogenesis of diabetic complications, the mechanisms involved remain uncertain. There are several theories as to how chronic hyperglycemia can lead to micro or macrovascular disease in diabetes, including the advanced glycation end product (AGE) theory. Evidence for the effect of AGE in the development of diabetic angiopathy is derived not only from a number of in vitro and in vivo studies exploring the role of AGE in different pathologies, but also from studies demonstrating significant improvements of features of diabetic complications by anti-AGE agents. Although it is well established that AGE are involved in the pathogenesis of diabetic complications, more studies are needed to elucidate the exact role of AGE in this area. The use of the "new" and "old" anti-AGE agents will help both in the study of the mechanisms involved and the therapeutic applications aiming at prevention or amelioration of diabetic complications that still constitute a major problem with a life-threatening impact for diabetic patients, worldwide.

Key words: Advanced glycation endproducts, AGE, Dietary AGE, Atherosclerosis, Diabetic microangiopathy, Diabetic macroangiopathy, AGE receptors, Anti-AGE agents

INTRODUCTION

Diabetes mellitus, especially type 2 diabetes, is increasing at an alarming rate and is considered as one of the main threats to human health in the 21st century, in both developed and developing nations. More than 150 million people currently have diabetes, and twice that number is at high risk of developing diabetes in the next 5-10 years, while type 2 diabetes in children and adolescents is considered an emerging health problem.

Most patients with diabetes develop microvascular disease, while macrovascular disease is associated with an increased morbidity and mortality from coronary, cerebrovascular and peripheral vascular events.

A large body of evidence emerging mainly from
the two landmark studies, the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), indicate that chronic hyperglycemia constitutes a major initiator of microvascular diabetic complications, but the exact mechanisms have not yet been fully elucidated.5-7

There are several, well-researched theories of how chronic hyperglycemia can lead to micro or macrovascular disease in diabetes including the advanced glycation end product (AGE) theory.7-10 Many in vitro and in vivo studies but also studies using anti-AGE agents have demonstrated that these chemically heterogeneous compounds are known to have a wide range of chemical, cellular and tissue effects implicated in the development and progression of diabetic complications.8-10

This review will outline the nature, formation and metabolism of AGE as well as evidence on their pathogenic potential in type 2 diabetes-related complications.

1. AGE SOURCES

AGE constitute a heterogenous group of molecules formed by the nonenzymatic reaction of reducing sugars, ascorbate and other carbohydrates with amino acids, lipids and nucleic acids and through lipid peroxidation as well.8-11 Although this process takes place continuously within the body during ageing, it is extremely accelerated in diabetes.8-12

It should be emphasized, however, that a large portion of these agents can be exogenous. Tobacco smoke has already been recognized as an important exogenous source of AGE.13 Recently, it has been found that diet, especially the modern Western diet, provides a relatively large portion of preformed AGE and AGE-precursors.8,10 The ways that food is processed for safety, conservation and improving taste, flavor and appearance lead to the generation of diverse unstable a-β-dicarbonyl derivatives of glyco- and lipoxidation reactions.14-17 Although, the exact nature of various diet-derived AGE derivatives has not yet been fully elucidated, recent studies showed that "N-carboxymethyl-lysine (CML) and methylglyoxal (MG) derivatives, which constitute products of protein and lipid glyoxidation, are present in most foods. A recent study, in which CML, was estimated in over 200 common foods, showed that AGE generation, although influenced by the content and type of nutrients (fats>proteins>carbohydrates), depended mainly on the specific conditions applied, such as cooking method, humidity, time and temperature used through food processing.18

Exogenously “offered” AGE are absorbed in the gastrointestinal tract (-10%) and delivered to the liver and to other tissues, 1/3 is excreted in the urine, and the remaining is involved in the AGE-related pathology in diabetes.19-25

2. AGE METABOLISM AND INTERACTIONS

Despite intensive investigation, the elucidation of the structure of specific AGE remains a problem. The different methods used in the various studies lead to nonconsistent and conflicting results. Till now, there is no ideal way to measure various AGE moieties. The currently used methods are HPLC, chromatography, fluorescence and Elisa.

The term AGE, while referring to non-reactive terminal products such as CML and pentosidine in most studies, also includes many reactive intermediates or AGE-precursors such as 1- or 3- deoxyglucosone, MG and their derivatives.8-10,26

Circulating AGE levels reflect the equilibrium between endogenous formation and catabolism, including tissue degradation and renal elimination, as well as the oral AGE intake.

At the tissue level, macrophages and other cellular systems endocytose and degrade AGE via receptor or non-receptor pathways, resulting in the formation of low molecular weight AGE peptides.8,10,26 These peptides undergo a variable degree of reabsorption and further catabolism in the proximal nephron and the rest is excreted in the urine. Therefore, effective elimination is dependent on normal renal function.8-10,26,27

At the cellular level, there are intracellular protective systems which also limit the accumulation of reactive AGE derivatives. For instance, MG is first converted by glyoxalase-I to S-D-lactoylglutathione
and then to D-lactate by glyoxalase-II.\textsuperscript{28}

The above homeostatic systems, however, can be overwhelmed in high AGE conditions such as diabetes and renal failure, especially when combined with increased dietary AGE intake.\textsuperscript{27,29}

AGE can cause tissue damage by two main pathways: they either form cross-links that disrupts the structure and function of short and long-lived proteins and lipids or they interact with specific and non-specific for AGE cell surface receptors, leading to altered intracellular events that induce oxidative stress and inflammation.\textsuperscript{8-10,26}

The AGE-receptor system, which includes, specific and non-specific for AGE receptors and a few soluble binding proteins, seems to play an important role in the AGE homeostasis. This system involves AGE-R1, a 50kD protein, involved in ligand endocytosis and processing, AGE-R2, a 80-90kD protein, involved in early signalling and AGE-R3, a 30-35kD protein, contributing to both removal and cell activation. There are also other important molecules such as RAGE, linked to cell activation mainly via oxidative stress induction, scavenger receptors, class A (MSR-A) and class B (MSR-B) and lysosome, involved in cellular uptake and degradation of AGE. As in the case for other receptors, the exact ligands to the AGE-receptors have not yet been fully elucidated.\textsuperscript{8-10,26}

3. AGE AND DIABETIC COMPLICATIONS

AGE have been considered as important pathogenic mediators in diabetes-related complications, conventionally grouped as micro- or macroangiopathy.

3.a. AGE and microangiopathy

The term diabetic microangiopathy involves a broad spectrum of dysfunctional changes in microvascular beds such as retinas and kidneys, and a wide range of tissues such as peripheral nerves and skin.

3.a.1. Nephropathy

Diabetic nephropathy is now a major cause of end-stage renal disease.\textsuperscript{7,30} Although genetic susceptibility plays a role in its pathogenesis, hyperglycemia has been linked to the pathogenesis of diabetic nephropathy, acting through many pathways including AGE formation and action.\textsuperscript{7,10,26}

AGE cross-links, with important matrix proteins such as collagen, lead to changes of both their structure and function which is restored by the administration of anti-AGE agents.\textsuperscript{31-33} Also, AGE interact with the renin-angiotensin system, another potential mechanism for initiating renal disease.\textsuperscript{34} In addition, AGE induce cytokines, adhesion molecules, chemokines, growth factors and oxidant stress production which are involved in the pathogenesis of diabetic nephropathy.\textsuperscript{35-39} The above data have been supported by various in vitro and in vivo studies.

In vitro, AGE receptors have been found in renal mesangial cells which bind AGE, resulting in overproduction of matrix proteins, changes in the expression of matrix metalloproteinases and protease inhibitors,\textsuperscript{40,41} induction of mesangial oxidative stress and activation of protein kinase C-\textbeta.\textsuperscript{35} Various types of preformed AGE-BSA, produced in cultured human mesangial cells, resulted in vascular endothelium growth factor (VEGF) and MCP-1 proteins secretion and apoptosis, events that were prevented by N-acetylcysteine, an antioxidant proposed as an anti-AGE agent.\textsuperscript{42}

In vivo, increased glomerular basement membrane, mesangium, podocytes and renal tubular cells in association with increased AGE deposition were found immunohistochemically in kidneys from normal and diabetic rats, rising with age and more rapidly with diabetes.\textsuperscript{42,43} In addition, short-term exogenous AGE administration in normal, non-diabetic animals was associated with increased production of basement membrane components (e.g. collagen IV), extracellular matrix regulatory factors (e.g. transforming growth factor-beta), all consistent with the findings of diabetic nephropathy.\textsuperscript{45,46} Furthermore, RAGE overexpression in diabetic mice resulted in increased albuminuria, elevated serum creatinine, renal hypertension, mesangial expansion and glomerulosclerosis compared to non-diabetic littermates,\textsuperscript{47} changes that were restored by pharmacological blockade of RAGE,\textsuperscript{48} while galectin-3 knock-out mice demonstrated a significant protection against diabetic nephropathy.\textsuperscript{49} In addition, anti-AGE agents (AGE inhibitors and AGE-breakers) have been shown to
diminish AGE accumulation in renal structures and also diabetic nephropathy in experimental diabetest.33,50,51

Human studies have shown increased CML, pyralline and pentosidine deposition in the renal tissue of diabetic subjects with or without end-stage renal disease, increasing in parallel with the severity of nephropathy, as well as a significant reduction of nephrin, an important regulator of the glomerular filter integrity.52,53 A diffuse upregulation of RAGE expression in podocytes, colocalizing with synaptopodin expression has been found in the glomeruli of patients with diabetic nephropathy.54

3.a.2 Retinopathy and eye complications

Diabetic retinopathy occurs in three fourths of all persons with diabetes after more than 15 years of the disease, and is considered as the most common cause of blindness.7,55 AGE have been involved in the pathogenesis of diabetic retinopathy by altering small vessel wall integrity and structure and by inducing cytokines, growth factors and increased oxidative stress.7-10,26,56-58

In vitro, retinal endothelial cells exposed to AGE overproduced VEGF through oxidative stress induction, PKC pathway activation and abnormal endothelial nitric oxide synthase (eNOS) expression.59,60 Retinal organ cultures showed an increased glyoxal induced CML formation in association with increased apoptosis and cell death, restored by anti-AGE agents and antioxidants.59

Increased AGE accumulation was also found in diabetic rats after 8 months of diabetes, in vascular basement membrane but also in the retinal pericytes.57 In addition, exogenous AGE-albumin administration in non-diabetic animals accumulated around and within the pericytes, colocalized with AGE receptors inducing retinal vessel wall thickening and loss of retinal pericytes.61,62

In humans, increased AGE accumulation distributed around blood vessels has been found in the retinal vessels of diabetics, increasing with the severity of retinopathy.63 Glycation of vitreous collagen was also found in vitreous from human donor eyeballs.64 In addition, studies using anti-AGE agents have further support the role of AGE in diabetic retinopathy.65-68

Increased levels of glycosylation products have also been found in cataract lenses,69-71 which have been associated with abnormalities in the Na-K-ATPase pump, leading to significant alterations in lens membrane integrity and function and cataract formation in diabetes, changes restored by pyruvate administration.72-74

AGE have also been linked to the changes associated with diabetic keratopathy through their effect in reducing corneal epithelial cell adhesion and spreading.72,73

Furthermore, glycation of the vitreal collagen fibrils leading to dissociation from hyaluronan and resultant destabilization of the gel structure has been associated with vitreous liquefaction and posterior vitreous detachment in diabetes.75-77

3.a.3. Neuropathy

Diabetic neuropathy is encountered in about half of all people with diabetes either as a polyneuropathy or mononeuropathy.7,78 Glycation of cytoskeletal proteins, through structural or functional changes of the nerve fibers, has been involved in the pathogenesis of diabetic neuropathy.79-82

In vivo, a reduction in sensory motor conduction velocities and nerve action potentials as well as in peripheral nerve blood flow has been reported in diabetic rats, which is prevented by pretreatment with AGE inhibitors.82,83 In addition, increased AGE accumulation has been described in cytoskeletal proteins of the sciatic nerve of diabetic rats which decreased after islet transplantation.84

Furthermore, increased AGE accumulation has been described in the cytoskeletal and myelin protein extracts of the sural and peroneal nerves of human subjects, distributed in the cytoplasm of endothelial cells, pericytes, axoplasm and Schwan interstitial collagens and basement membranes of the perineurium cells of both myelinated and unmyelinated fibers correlated with the myelinated fiber loss.85,86 In addition, AGE accumulation in the vasa nervorum has been linked to segmental demyelination by causing vascular abnormalities.87
3.4. Dermopathy

Various studies have shown an increased accumulation of various glycosylation products in the skin in diabetes which alters its physicochemical structure, leading to diabetes skin-related disorders. Furthermore, AGE have been implicated in the pathogenesis of delayed wound healing in diabetes. 

3. b. AGE and macroangiopathy

The term macrovascular disease in diabetes includes atherosclerosis and increased stiffness of the arterial wall mediated by the interplay of various factors including AGE.

In vitro studies have shown that AGE form intra- and intermolecular cross-links with matrix proteins in the vascular wall increasing vessel rigidity, trapping lipoproteins within the arterial wall and disrupting its clearance.

Glycated LDL has also been shown to stimulate production of plasminogen activator inhibitor-1 (PAI-1) and to reduce generation of tissue plasminogen activator (tPA) in cultured human vascular endothelial cells. Glycated HDL has also been linked to decreased ability to prevent monocyte adhesion to aortic endothelial cells, while lipoprotein(a) glycation has been shown to increase PAI-1 production and decrease t-PA generation. AGE interaction with endothelial cell receptors has shown to induce increased vascular permeability, procoagulant activity, migration of macrophages and T-lymphocytes into the intima and impairment of endothelium-dependent relaxation.

In vivo, an increased AGE deposition has been described in aortic atherosclerotic lesions, correlated with the degree of atheroma, events which were restored by using anti-AGE agents.

An increased AGE deposition has also been found in the atherosclerotic plaque in vessels from diabetic patients and in the radial artery wall of chronic renal failure patients with or without diabetes. In addition, an increased tissue AGE accumulation and AGE receptors with a similar distribution pattern associated with an increased aortic stiffness have been found in human aortas obtained from post-mortem examination of diabetic subjects.

Furthermore, increased circulating AGE levels and increased vascular tissue AGE deposition associated with impaired endothelium dependent and endothelium-independent vasodilatation and increased arterial stiffness have been found in diabetic patients, restored by the administration of anti-AGE agents.

4. ANTI-AGE STRATEGIES

Several approaches seeking to reduce AGE interactions, either by inhibiting AGE formation, blocking AGE action or breaking pre-existing AGE cross-links, have been explored.

Glycemic Control. Hyperglycemia has been linked to increased AGE formation, making obvious that the achievement of a good metabolic control can reduce the total body AGE pool. Indeed, lower levels of AGE and decreased collagen-linked glycosylation have been demonstrated in diabetic rats with good compared to bad metabolic control. In addition, lower skin collagen glycosylation has been found in a large group of diabetic patients under intensive versus conventional treatment, in the Diabetes Control and Complications Trial.

Dietary modification. Diet has been considered as an important exogenous source of AGE making obvious that dietary modification in terms of consuming diets with low AGE content can decrease the total body AGE pool and AGE-related pathology. Antioxidants. Although various antioxidants have been proposed as anti-AGE agents, further studies are needed in order to establish the effectiveness of this treatment in reducing AGE levels.

Anti-AGE agents. The first class of those agents involved the inhibitors of AGE formation which act by inhibiting post-Amadori advanced glycation reactions or by trapping carbonyl intermediates and thus inhibiting both advanced glycation and lipid oxidation reactions. Aminoguanidine, ALT-946, 2-3-Diaminophenazine, thiamine pyrophosphate, benfotiamine and pyridoxamine, ORB-9195 constitute known representatives of this group of agents. The second class of those agents involved the AGE breakers, which “break” pre-ac-
cumulated AGE or existing AGE cross-links, leading to the elimination of the smaller peptides through urine. PTB (N-phenylthiazolium bromide)\textsuperscript{131} and ALT-711 are the best known representatives of this group of agents.\textsuperscript{33,132}

**Other agents.** Recently, it has been shown that antihypertensive drugs such as losartan, olmesartan, and hydralazine, seem to inhibit AGE formation.\textsuperscript{133-135}

**CONCLUSION**

It is well established that AGE are involved in the pathogenesis of diabetic complications. However, more studies are needed to elucidate the exact role of AGE in this area. The use of the “new” and “old” anti-AGE agents will help both in the understanding and the treatment of diabetic complications that still constitutes a major problem with life-threatening impact worldwide.

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