ACTA SCIENTIFIC NUTRITIONAL HEALTH

Volume 3 Issue 5 May 2019

Review Article

Non-Enzymatic Glycation and Anti-Glycation Strategies

Rakshmitha Marni*

Department of Biotechnology, GITAM Institute of Sciences, GITAM University, Visakhapatnam, India

***Corresponding Author:** Rakshmitha Marni, Department of Biotechnology, GITAM Institute of Sciences, GITAM University, Visakhapatnam, India.

Received: March 29, 2019; **Published:** April 26, 2019

Abstract

Reducing sugars react non-enzymatically with free amino groups of proteins to form a Schiff's base, which undergoes a series of irreversible reactions to form an Amadori product and finally yield advanced glycation end products (AGEs). Formation of AGEs occurs with normal aging, however their production accelerates during hyperglycemia. Diets rich in sugars and lipids and food-processing methods such as deep fat frying and high-temperature cooking contributes to serum and tissue AGEs in addition to endogenous AGEs formed in the body. AGEs contribute to the post-translational modifications of several proteins including collagen, eye lens crystallins, hemoglobin, and extracellular matrix proteins. AGEs interaction with cell-surface receptor, RAGE, activates several intracellular signaling that manifest in aberrant cellular responses such as increased production of reactive oxygen species, inflammation, and apoptosis. Elevated levels of AGEs are associated with adverse outcome in diabetic, cardiovascular, and neurodegenerative complications. Anti-AGEs, AGE-breakers, antioxidants and diet low in sugars and oils help to reduce AGEs levels and AGE-mediated pathophysiological complications.

Keywords: Glycation; AGEs; NEG

Introduction

Proteins undergo several post-translational modifications (PTMs) and such modifications are often associated with altered structure, function, and half-life of proteins. Non-enzymatic glycosylation (NEG) of proteins is one of the predominant PTM that involves the reaction of reducing sugar moieties with free amino groups of amino acids. NEG of proteins is a normal process during aging. However, it accelerates during hyperglycemia and hypercholesteremia etc. NEG, in addition to adversely affecting the physiological properties of the susceptible proteins, it also elicits aberrant cellular signaling. The sequelae of events in NEG begin with a reaction between reducing sugars (e.g.: glucose or fructose) and positively charged amino acids (e.g.: lysine or arginine). Intermolecular nucleophilic attack anomeric carbon of the glucose by lysine (or arginine) in proteins results in the formation of Amadori product. Amadori products undergo successive displacements of the carbonyl group along the carbon skeleton of reducing sugar, which results in the formation of a variety of dicarbonyl intermediates (e.g.: Methylglyoxal, glyoxal). The dicarbonyls undergo a series of intramolecular rearrangements and form precursors for advanced glycation end products (AGEs). The term AGEs describes any protein bound adduct detected after formation of the initial Schiff base/Amadori product. AGEs may result from a one-step conversion from Amadori product (formation of carboxymethyl lysine), or multiple reactions (formation of pentosidine). The process of NEG takes days to weeks whereas formation of AGEs may take weeks to years. These classical reactions are popularly known as Maillard reaction or non-enzymatic browning reaction. Though AGEs are a heterogeneous group of complex compounds, they share some common properties such as exhibiting color, fluorescence, insolubility, and formation of cross-links, which help them for their characterization. Among several of them, carboxymethyl lysine (CML), pentosidine, and fluorine and are well characterized AGEs. In recent times, NEG of lipids, nucleic acids are also reported [1].

Sources of AGEs

The Maillard reaction/browning reactions have utmost importance for exogenous AGE formation. It induces browning of heated foods, which ultimately has an effect on both nutritive quality and taste of the food. Processed foods like bread, biscuits, red meat, breakfast cereals, fried eggs, and many beverages undergo NEG during the cooking process, which elicits the accumulation of AGEs. The AGE formation can be affected by the concentration of reducing sugar, nutritive composition, duration of the food processing, heating temperature, and method of heating [1,2]. In fact, heat processed meat derivatives such as grilled chicken, fried bacon, roasted beef contain more AGEs than boiled carbohydrates for a longer time [1,3].

AGEs are also formed endogenously as a result of the body's normal metabolism. However, *in vivo* formation of AGEs accelerates during certain pathological conditions like hyperglycemia and hyperlipidemia. Beside NEG reaction another well-known pathway studied is "Sorbitol aldose reductase pathway" well known as "polyol pathway" which involves sequential enzymatic reactions. Presence of glucose in larger amounts (as in diabetes mellitus) leads to activation of the polyol pathway where glucose is converted to sorbitol by aldose reductase and then to fructose by the action of sorbitol dehydrogenase. Fructose is more reactive than glucose and excess fructose contributes to the formation of dicarbonyls and finally AGEs [4]. Other alternative pathways like "Hodge pathway" in which formation of AGEs take place by auto-oxidation of Amadori products and cleavage of dicarbonyls compounds from Schiff's base known as "Namiki pathway" and by autoxidation of monosaccharide's (glucose, fructose, xylose) known as "Wolff pathway" apart from this degradation of disaccharides unattached to a protein also involve in AGE formation [5]. The Intake of Dietary AGE-rich foods contribute to the increase in endogenous AGE pool and serum AGE levels by affecting the endogenous AGE metabolism. In general, *in-vivo* AGE formation is a homeostatic process where formed AGEs are degraded by AGEs-receptor 1 (AGER1). The rate at which AGEs are formed depends on several factors such as the concentration of the substrate, the glycating agents, the half-life of the proteins, the availability of catalytic compounds, reactive oxygen species (ROS), and redox balance. Endogenous AGEs accumulation or load of exogenous AGEs contributes to organ damage over a period of time. Recent evidence suggests that AGEs contributes to cataract development in elderly patients with diabeticmellitus [6].

Intake of AGE-rich diets contributes to the endogenous serum AGE levels where they undergo cross-linking with several proteins present in the body including plasma proteins, hemoglobin, collagen, elastin, eye lens crystallins, and immunoglobulin. Proteins that undergo NEG and form AGEs become non-functional and toxic. In addition to endogenous AGEs that are formed by cellular metabolism, dietary-AGEs also have a significant role in eliciting pathogenesis of various AGE related diseases by exerting their effect through activation of various cell signaling cascades via Receptor for advanced glycation end products (RAGE). AGEs largely present in heat-processed foods and are known to contribute to increased oxidative stress and inflammation. The role of dietary AGEs in human health and disease was ignored since it was assumed that they are poorly absorbed. Consumption of AGE-rich diets is associated with the incidence of kidney disease whereas dietary restriction of AGEs improved kidney dysfunction [7]. Dietary AGEs correlate with circulating AGEs (CML and methylglyoxal) and are markers of oxidative stress in healthy subjects [8]. Restriction of dietary AGEs helps in combating chronic kidney disease. The dietary sources rich in anti-oxidants prevent glycation and accumulation of AGEs. Feeding green tea extract prevented AGEs-related collagen cross-linking and the formation of fluorescent AGEs in C57BL/6 mice [9]. Epigallocatechin-3-gallate exhibited promising *in vitro* antiglycation potential by acting as reactive carbonyl scavenger and antagonize AGE-induced pro-inflammatory changes [10]. Foods high in carbohydrates have the lowest amount of AGEs.

The physiological manifestation of AGEs: Under normal conditions, NEG occurs at a slow rate over a lifetime, but occurs more rapidly in clinical conditions such as hyperglycemia and hypercholesteremia. Accumulation of these products leads to worsening of many diseases like Diabetes, cardiovascular diseases, neurodegenerative diseases [11,12]. Expression of RAGEs is low under normal physiological conditions, but they overexpress in response to oxidative stress and inflammation. AGE-RAGE axis elicits pro-oxidant and pro-inflammatory events through several intracellular signaling cascades. AGE-RAGE axis stimulates aberrant NF-KB signaling, induces apoptosis via p53-Bax expression, and generation of ROS via activation of NADPH oxidize [13]. ROS generated during AGE-RAGE interaction promote an array of proinflammatory activities. Activation of AGE-RAGE axis induces JAK/STAT signaling [14] that elevates expression of ZEB2 [15], TGF-β, CTGF (connective tissue derived growth factor), PDGF (Platelet-derived growth factor) and many cytokines, whose

levels are increased during diabetic nephropathy [14]. Activated AGE-RAGE axis, which propagates cellular dysfunction by a variety of means that may imply in a number of patho-physiological conditions.

The progressive accumulation of AGEs in the eye lens contributes to irreversible glycation of lens crystalline and affects protein-protein and protein-water interaction that leads to decreased lens transparency and cataract [16,17]. Damage to the peripheral nerve fibrils is also accounted for by the accumulation of AGEs. It was reported that AGEs may probably interfere with the axonal transport by modifying the neuron filament and tubulin thereby indicating the role of AGEs in diabetic neuropathy [18].

AGEs accumulate in the various parts of the nephron in patients with diabetes mellitus. Glomerular accumulation of AGEs stimulates MCP-1 in glomerular mesangial cells, which eventually leads to the accumulation of extracellular matrix and renal fibrosis [19,20]. It was demonstrated that higher levels of RAGE expression are seen in the podocytes of diabetic human and rat kidney sections [21]. AGEs induce epithelial-mesenchymal transition of podocytes that compromises glomerular permselectivity and results in proteinuria [15]. The analysis of AGE levels in the periphery nerve was shown to have increased in CML and 3-DG in streptozotocin-induced diabetic rats [22]. In another study, it was reported that increased CML, CEL, MG-derived hydroimidazolone and fructosyl-lysine in the diabetic nephropathy rat model [23]. AGE-glycation leads to PTM of many proteins in nerve fibers like tubulin and neurofilament in the axons and myelin protein in Schwann cells was shown to be glycated. Collagen, fibronectin, and laminin in the basement membrane and extracellular matrix were modified by AGEs [18,24]. Accumulations of AGEs due to endogenous or exogenous factors contribute to severe changes. In the cardiovascular system where crosslinking of elastin and collagen resulting in myocardial and arterial stiffness which further leads to the development of cardiac fibrosis [25]. Diabetes induced mice with a control diet and normal mice with AGE rich diet for 12 weeks have shown increased levels of AGEs, TNF-α, IL-6, LDL, ROS, and RAGE expression, and decreased superoxide dismutase (SOD) levels [26]. Another study revealed that AGEs also exert cardiovascular damage by glycation of low-density lipoproteins (LDL), which affect the cellular uptake of LDL. The glycated LDL from cross-links with collagen and not taken up by the cell and accumulates. Macrophages uptake of these modified LDL lead to foam cell formation, which serves as the hallmark of early stage atherosclerotic lesion formation [27,28]. Higher dietary AGE intake was shown to be deleterious on wound closure and angiogenesis. Lowering of dietary AGEs is found to be effective in promoting wound healing, epithelization and granulation tissue depositions in obese type 2 diabetic mice [29].

Anti-glycation strategies

Anti-AGEs and AGE-breakers: Amino guanidine acts as a nucleophilic trap for reactive carbonyl intermediates thus prevents the advanced and irreversible steps of NEG [30]. 2-isopropylidenehydrazono-4-isothiazolidine-5-acetanilide (OPB-9195) is another synthetic anti-AGE compound designed to trap reactive carbonyl intermediates [31]. Pyridoxamine scavenges ROS and inhibits post-Amadori stages of AGE formation. Oral administration of pyridoxamine to diabetic rats attenuated CML accumulation [32]. Dietary supplementation of arginine has been employed as an anti-AGE agent. In a long term study of 22 women with polycystic ovarian disease (PCOS) given metformin treatment for 6 months reduced of serum AGE levels [33]. A recent screening of natural compounds has shown to have potential effects on AGE degradation. The root extracts of Scutellaria alpina and S altissima were known to have anti-oxidant property and are demonstrated to be highly effective anti-glycation activity [34].

AGE-breakers are small molecules that able to break the Amadori products or AGE-cross-links. Synthetic molecules such as ALT-946 and ALT-711, N-phenacetylthiazolium bromide (PTB) have been developed chemically to break Maillard reaction cross-links. Diabetic rats treated with ALT-711 prevented glycation of collagen. ALT-711 reduced TGF-β expression and prevented epithelial-myofibroblast transdifferentiation in diabetic rats suggesting that AGE cross-link breakers are possible options to target fibrosis in diabetic settings [14]. Interestingly AGE-breakers also reduced RAGE expression in diabetic rats. After injection of single AGE-rich meal, the *in-vitro* exposure of serum fractions was found to have increased covalently-linked adducts of AGE with fibronectin, which was blocked by amino guanidine and administration of OPB-9195 lowered glycated albumin levels in rats [31]. As it was previously noted that AGEs elicit their effects via activation of RAGE, therefore, employing Anti-RAGE neutralizing antibodies are one of the strategies to combat AGE mediated effects [35].

Antioxidants: Early reactions of NEG accelerate during oxidative stress conditions, particularly in conversion of Schiff's base to Amadori products. As the rate of AGEs formation increases in oxidative stress condition, therefore antioxidants offer intervention by preventing AGEs formation. Glutathione is a natural antioxidant and it was shown to combat oxidative stress and deaccelerate rate of non-enzymatic glycation reactions. Inhibitors of aldose reductase such as statil, curcumin prevented NEG in diabetic rats. In addition, antioxidants including lipoic acid, vitamin E and ubiquinol prevented the demand for glutathione by serving as redox regulators and prevented AGEs mediated cellular toxicity. Natural antioxidants such as vitamin C and E, α-tocopherol, niacinamide, pyridoxal, lipoic acid and chelating agents such as sodium selenite, selenium yeast, riboflavin, zinc, and manganese has been shown

to inhibit glycation [36-39]. Dietary supplementation of alpha-lipoic acid prevented the glycation of collagen in fructose-fed rats [36]. Vitamins C and E and a combination of N-acetylcysteine with taurine and oxerutin were shown to elicit *in vivo* anti-glycation potential [40]. It was reported that N-acetyl cysteine inhibited ROS dependent NF-kB activation thus preventing AGEs mediated aberrant cellular events [40]. In addition to antioxidants, statins such as Pravastatin, Atorvastatin, and Cerivastatin offer protection against AGEs mediated injury in several ways including suppression of RAGE expression, by preventing VEGF and NF-kB expression [41-43].

Food-processing recommendations: It is recommended to avoid heat-processed foods and completely processed foods that were high in protein and fat. Dairy-based processed foods and beverages contain large amounts of AGEs. A diet heavy in AGEs results in proportional elevations in serum AGE levels in diabetic patients. Several spices including curcumin, cinnamon, and ginger were shown to inhibit glycation *in vitro*. Natural flavonoids, such as lutein, quercetin, and rutin were shown to inhibit AGE formation [10,44]. ProcyanidinB2 prevented accumulation of CML in the glomerular region. It is interesting to note that cinnamon and procyanidin B2 [44], ellagic acid [45] also possess anti-glycation potential. Foods like breakfast cereals, legumes, vegetables and fruits that are rich in carbohydrates contain few AGEs. Dry heat cooking methods including grilling, pan-frying, broiling, roasting, baking, and deep fat frying accelerate AGEs formation. AGEs formation during cooking can be significantly reduced by cooking food at low temperatures, cooking for short durations, using moist heat methods and by using acidic ingredients like lemon juice.

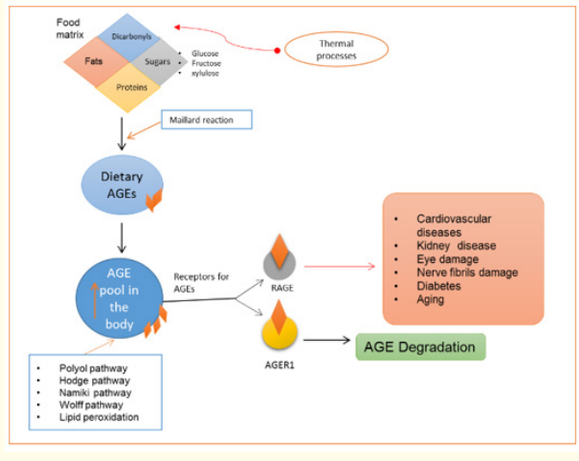

Figure 1: Schematic representation of enhanced AGE levels in-vivo and their health complications.

AGEs- Advanced glycation end products, RAGE-Receptor for advanced glycation end products, AGER1-Advanced glycation end products receptor 1.

Conclusion

Regulation of glucose homeostasis has been best option combating NEG and AGE formation. It is evident that both dietary AGEs and tissue AGEs contribute to the pathological complications. Therefore, evaluation of efficacy and safety of anti-AGEs compounds including AGEs blockers/cross-link breakers, and sRAGE, could be a better therapeutic choice for combating AGE mediated complications (Figure 1). Successful accomplishment of anti-AGE therapy could be a part of a regimen for subjects with AGES-mediated diabetic renal complications. Besides regulating blood glucose to the normal range, doing physical activities, intake of antioxidants and anti-AGE compounds rich diet offer protection from AGEs mediated complications. As said prevention is better than cure, avoiding sugar processed foods, fatty meats, full-fat dairy products, and solid fats dietary intake of AGEs can be restricted.

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Volume 3 Issue 5 May 2019