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Advanced Glycation End Products: Key Mediator and Therapeutic Target of Cardiovascular Complications in Diabetes

Advanced Glycation End Products in Diabetic-Cardiovascular Complications

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Abstract

The incidence of type 2 diabetes mellitus is growing in epidemic proportions and has become one of the most critical public health concerns. Cardio-vascular complications associated with diabetes are the leading cause of morbidity and mortality. The cardiovascular diseases (CVD) that accompany diabetes include angina, myocardial infarction, stroke, peripheral artery disease, and congestive heart failure. Among the various risk factors generated secondary to hyperglycemic situations, advanced glycation end products (AGEs) are one of the important targets for future diagnosis and prevention of diabetes. In the last decade, AGEs have drawn a lot of attention due to their involvement in diabetic pathophysiology. AGEs can be derived exogenously and endogenously through various pathways. These are a non-homogenous, chemically diverse group of compounds formed non-enzymetically by condensation between carbonyl groups of reducing sugars and free amino groups of protein, lipids, and nucleic acid. AGEs mediate their pathological effects at the cellular and extracellular levels by multiple pathways. At the cellular level, they activate signaling cascades *via* the receptor for advanced glycation end products (RAGE) and initiate a complex series of intracellular signaling resulting in ROS generation, inflammation, cellular proliferation, and fibrosis that may possibly exacerbate the damaging effects on cardiac functions in diabetics. AGEs also cause covalent modifications and cross-linking of serum and extracellular matrix (ECM) proteins; altering their structure, stability, and functions. Early diagnosis of diabetes may prevent its progression to complications and decrease its associated co-morbidities. In the present review, we recapitulate the role of AGEs as a crucial mediator of hyperglycemia-mediated detrimental effects in diabetes-associated complications. Furthermore, this review presents an overview of future perspectives for new therapeutic interventions to ameliorate cardiovascular complications in diabetes.

Key Words: Type 2 Diabetes Mellitus; Cardiovascular complications; Hyperglycemia; Advanced glycation end products; Reactive oxygen species; Oxidative stress; Endothelial cells; Receptor of advanced glycation end products; Anti-AGEs strategies.

Core Tip: Cardiovascular diseases (CVD) in type 2 Diabetes Mellitus (T2DM) impose a clinical and an economic burden on the healthcare system. Early diagnosis of diabetes may prevent its progression to complications and decrease its associated co-morbidities. The present manuscript reports the clinical relevance of estimating advanced glycation end products (AGEs) in diabetes. The deleterious effects of AGEs include many important biochemical reactions central to the development and progression of cardiovascular complications in diabetes. Therefore, AGEs are one of the important targets for future diagnosis and prevention of diabetes. The epidemiology of CVD in diabetes, AGEs as a crucial mediator of diabetic-CVD, and an overview of different strategies for countering the accumulation of AGEs is discussed along with new therapeutic interventions to ameliorate their effects.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a cluster of metabolic disturbances consequent to non-utilization of glucose due to insufficient production/secretion of insulin or its resistance. T2DM poses a major threat to global health. The number of people with T2DM is increasing at an alarming rate and has become one of the leading causes of death worldwide. The upsurge is corresponding with rising obesity, aging populations, increasing urbanization, calorie dense diets, economic development, and reduced physical activity. The global prevalence of diabetes as described by the International diabetes federation (IDF) in 2021 was estimated to be 536.6 million (10.5%) and it is projected to reach 783.2 million (12.2%) by 2045 [1]. Prevalence is expected to be higher in urban areas compared to rural ones. The estimated global cost of diabetes is slated from 966 billion USD in 2021 to rise to 1,054 billion USD by 2045 [1,2]. Consequently, T2DM imposes both a clinical and an economic burden on the health care system.

Diabetes Mellitus is a complex pathophysiological process associated with several disabling and life-threatening health problems. Since diabetes mellitus basically affects blood vessels, it can affect almost any part of the body. People with diabetes are at risk of developing several complications affecting the heart, eyes, kidneys, and nerves. Vascular dysfunction is the single most serious consequence of long-standing diabetes [3, 4] resulting in debilitating morbidity and mortality due to cardiovascular diseases (CVD) [5, 6]. The CVD that accompanies diabetes includes stroke, myocardial infarction, peripheral artery disease, and coronary thrombosis [7].

Early diagnosis of diabetes may prevent its progression to CVD and decrease its associated co-morbidities. Persistent hyperglycemia is considered to be an important factor in the development and the progression of diabetic associated complications and exact mechanism of the deleterious effects of hyperglycemia on the onset of diabetic complications is still being explored [8]. Numerous hyperglycemia-induced mechanisms have been hypothesized to account for vascular complications in T2DM. These include the hexoamine pathway, poly ADP ribose polymerase (PARP) activation, protein kinase C (PKC) activation, aldose reductase-mediated polyol pathway, and enhanced formation of advanced glycation end products (AGEs) [9-11]. Among these, the AGE-mediated pathways have been explored in the last decade because of mounting evidence that AGE accumulation is the crucial factor in the progression of diabetic complications [12, 13]. Advanced glycation end products are heterogeneous compounds resulting from non-enzymatic reactions of reducing sugars with other biomolecules such as lipids, proteins, and nucleic acid. This non-enzymatic glycation of proteins, lipids and nucleic acids is a slow and complicated process depending on the relative concentrations of the reactants. The moderate presence of AGEs has been notice in healthy individuals whereas, its formation increased under hyperglycemic conditions [14]. The severity of complications in T2DM through AGEs corresponds with the quantum of hyperglycemia and varies with the structural and functional changes generated in most macromolecules. Also, AGEs interact with their receptors namely the receptor of advanced glycation end products (RAGE), and trigger the activation of

multiple signals that can affect cellular functions and metabolism through up-regulation of inflammation and oxidative stress [15, 16].

The importance of AGEs in diabetic-CVD is corroborated by the fact that the serum level of AGEs in T2DM-CVD patients is higher compared to diabetic patients without CVD [17, 18]. Studies have shown the association of AGEs with the prevalence as well as pathophysiological mechanisms of CVD in T2DM [19-21]. Jia *et al*, found that the tissue level of AGEs was independently associated with cardiac systolic dysfunction in T2DM patients with heart failure compared to T2DM patients without heart failure [22]. *In vitro* studies have shown that treatment of cardiomyocytes with AGEs for 24 h significantly reduces calcium transient in cells due to increased reactive species production [23]. Elevated serum AGEs predicted increased mortality due to CVD in Finnish diabetic women who were followed up for 18 years [24]. In a recent review article by Dozio *et al*, the involvement of glycation in cardiovascular remodeling causing molecular, cellular, and interstitial changes in the heart and vessels through different mechanisms has been demonstrated [25]. Recently a cross-sectional study carried out by De la Cruz-Ares *et al* in 540 subjects, AGEs levels and intima-media thickness of carotid arteries were consistently observed to be higher in CVD patients with T2DM [26]. Even Ninomiya *et al*, have highlighted the importance of AGEs as a screening marker of atherosclerosis [27]. The AGE-RAGE axis further activates the pathological inflammation in plaques and atheromas [28]. Ren *et al*, identified the inhibition of prostacyclin in endothelial cells by AGE-RAGE system, which, promotes the formation of plasminogen activator inhibitor 1 (PAI-1) contributing to the stabilization of thrombus formation by inhibiting the fibrinolytic activity [29].

Therefore, this review focuses on summarizing the clinical relevance of AGEs in CVD development and progression in T2DM. Different anti-AGE strategies are also being discussed that may become potential candidates for future preventive and therapeutic strategies in diabetic-CVD.

EPIDEMIOLOGY OF CVD IN DIABETES

Current trends in the epidemiology of cardiovascular diseases (CVD) in type 2 diabetes mellitus present an underlying connection between chronic and uncontrolled diabetes and vascular complications [30]. Diabetes poses a major risk for the development of CVD and diabetes-associated mortality [5]. Prevalence of coronary artery diseases, peripheral vascular diseases, and carotid artery has been observed in different macro-vascular complications in diabetes [31]. Numerous epidemiological studies suggested that diabetes can accelerate atherosclerosis and increase the incidence of heart attacks and strokes [31, 32]. T2DM carries a two- to six-time higher risk of heart failure than non-diabetic patients and accounts for more than 50% of deaths in T2DM patients [6, 33, 34]. The CVD is a major comorbidity affecting about one-third of all people with diabetes. A cohort study carried out on 1.9 million people by Dinesh *et al*, identified T2DM as a significant risk factor for CVD, including stroke, heart failure, atherosclerosis, and myocardial infarction [35]. Moreover, type 2 diabetic patients are also prone to various cardiovascular risk factors, such as hypertension, dyslipidemia, and obesity that can directly promote the occurrence of cardiovascular complications in T2DM [36, 37].

A cohort study carried out by Shah *et al*, demonstrated that the occurrence of peripheral artery diseases and heart failure was higher in T2DM by 16.2% and 14.7% respectively [33]. Another cohort study carried out by National Health and Nutrition Examination Survey demonstrated that diabetes increases the risk of stroke by 26.3%, hemorrhagic stroke by 50%, and ischemic stroke by 50% [32, 38]. American heart report of 2014 revealed a risk of heart failure of 40% in T2DM patients compared to non-diabetics [39]. A prospective study showed that angina, coronary angioplasty, myocardial infarction, and congestive heart failure were among the predictors of all-cause mortality in T2DM [40]. A systematic review by Vaidya *et al*, has shown that approximately 15-81% of T2DM have at least one cardiovascular complications [41]. Results from the study by Thomas *et al*, confirmed that CVD embodies a substantial burden on the treatment of T2DM at both patient and population levels [42]. On average patients treated for both CVD and T2DM resulted in an additional cost ranging from $3,418 to $ 9,705 compared to T2DM alone. Given the substantial economic and health burden of CVD in T2DM

patients, there is a need to understand the mechanism of T2DM-CVD relationship and early diagnosis of T2DM to prevent its devastating complications.

DIFFERENT PATHWAYS FOR AGES FORMATION

Advanced glycation end products (AGEs) are chemically modified complex group of heterogeneous molecules formed either exogenously or endogenously by different pathways specifically, Maillard reaction, polyol pathway, and oxidation reactions elaborated in Figure 1. Maillard reaction was first described in 1912 by French Scientist Louis Camille Maillard as "browning reaction" due to the associated yellow-brown color change when reducing sugar was heated with amino acid [43]. The AGEs formed through Maillard reaction secondary to hyperglycemic condition is under intense investigation since a positive correlation with vascular complications like CVD, retinopathy, neurodegenerative diseases and other parameters of ageing [44-46]. Maillard glycation reaction is different from enzymatic N-/O-linked glycosylation of proteins since they are produce cross-linked products obtained from spontaneous and non-enymatic action of reducing sugars or their derivatives on other molecules, altering the structure and function of important cellular and extracellular components [47, 48]. In healthy individuals AGEs are formed very minimally which are cleared efficiently from the system. Formation and accumulation of AGEs becomes more rapid and pronounced under hyperglycemic conditions, oxidative stress, inflammatory conditions, and obesity [9, 16]. AGE levels are higher in aged individuals, due to either overproduction or slower clearance indicative of their pathophysiological implications [49, 50]

Accrual of AGEs is a multi stage process starting with covalent binding of functional groups of monosaccharide's to free amino groups of proteins, lipids, and nucleic acids forming labile reversible Schiff base intermediates under hyperglycemic environment. This reaction is reversed if the hyperglycemia abates soon enough. The initial Schiff base transforms over a period of days to a ketoamine, called Amadori's product. The Amadori products are more stable, but the reaction is still reversible. The most well recognized Amadori's product is glycated haemoglobin, widely used as a reliable

marker of glycemic control. Amadori's products can be degraded into a variety of dicarbonyl compounds like 3-deoxy-glucosone, glyoxal and methyl-glyoxal, which can further react with proteins to form intermediate glycation products. Yellow brown colored irreversible AGEs are formed after a sequence of chemical modifications including dehydration, oxidation, and fragmentation reactions (Figure 1). These spontaneous rearrangements are normally slow, often taking months to years. Nevertheless, the presence of oxidative stress, metal ions, and other catalysts can substantially increase the post-Amadori formation of AGEs. They are very stable and accumulate inside and outside the cells and some of them have fluorescent properties [9, 12, 16].

Besides Maillard reaction, other pathways like Hodge pathway, Namiki pathway and Wolff pathway can also result in AGE formation, through autoxidation interactions of Amadori products, monosaccharides (glucose, fructose, ribose and glyceraldehyde) with amino acids and lipids [16, 51, 52, 53]. Besides monosaccharides the reactive products formed during glycolysis can also form AGEs by attacking proteins and other components. Some of the important glycolytic intermediates indentified in AGEs formation are glyoxal, methylglyoxal, glucose-6-phosphate, triose phosphates glyceraldehydes-3-phosphate and dihydroxy- acetonphosphate and 3-deoxyglucosone [54, 55]. Auto-oxidation of glucose, reaction between glycolipid and arginine/lysine has also resulting in AGEs formation through glyoxal and methyl-glyoxal production [56, 57]. The Polyol pathway where, enzymatically formed metabolites of glucose like sorbitol and fructose also contributes significantly to AGEs formation [58, 59]. The free ribose formed during the degradation of nucleic acid also represents the main source of pentosidine formation [60].

Also, sugars vary in their susceptibility to the Maillard reaction, where D-glucose is less reactive and D-fructose is more reactive sugar as demonstrated in both thermally processed food and *in vivo* conditions [53, 61, 62]. Temperature also has a significant effect on early glycation product formation, where high temperature (120-180^{o}C) accelerates

the Maillard reaction in processed food, and the same reaction for amadori product formation *in vivo* conditions require much longer time [63].

Exogenous formation of AGEs through glyco-oxidation and lipo-oxidation reactions formed from heating food at high temperature and chemical processing, tobacco smoke components and other pollutants also contributing to the chemical load of AGEs. Blood and tissue AGE levels have been consistently observed to be higher in smokers and in patients on high AGEs diets compared to non-smokers and controls on low AGE diets [64, 65, 66, 67]. Ingestion of exogenous AGEs has been shown to exacerbate diabetic complications like CVD in animal models, hence their role needs further exploration [68, 69].

TYPE OF ADVANCED GLYCATION END PRODUCTS

Due to variety of precursors and numerous pathways of non-enzymatic reactions, the AGEs are diverse in their chemical structure and properties. AGEs comprise a large number of chemical structures like N-carboxy-methyl-lysine (CML) , pyrraline, pentosidine, cross-linked AGEs include GOLD [glyoxal-derived lysine dimer, 1,3-di(*N*_-lysino imidazolium salt], MOLD [methylglyoxalderived lysine dimer, 1,3-di(*N*_-lysino)-4-(methyl-imidazolium salt], DOLD [3-deoxyglucosone-derived lysine dimer, 1,3-di(*N*_-lysino)-4 (2,3,4-trihydroxybutyl)imidazolium salt], *etc*.[16,70-72]. The best biochemical and immunohistochemically characterized AGEs found in humans are pentosidine, carboxyl methyl lysine and methylglyoxal, which accumulate and can potentially be used as biomarkers [73, 74]. The CML is the most well characterized AGEs demonstrated in diabetic patients with CVD [75]. Structure and function of matrix proteins is modified with variable loss of function due to the aggregation of these adducts. Some of these AGEs have native fluorescence which can be used for their identification and quantification.

AGEs AND DIABETIC-CARDIOVASCULAR-COMPLICATIONS

Advanced glycation end products formed secondary to hyperglycemic conditions are gaining prominence as the underlying mechanism of CVD complications in T2DM. Diabetics are known to have 20-30% higher circulating AGEs compared to controls whereas, diabetics with CVD complications have up to 40-100% higher levels of AGEs [17, 76]. The AGEs remain significant high even after correction of variables such as duration of diabetes, sex, and age in T2DM patients with complications compared to those without complications [77, 78]. Statistical analyses have also shown the association of AGEs level with the development and severity of atherosclerosis in diabetic patients [79, 80]. Clinical reports have indicated that serum AGE levels can act as important marker or predictor of heart failure and CVD mortality in T2DM since their deposition has been detected in atherosclerotic plaques heart muscles [81, 82].

The deleterious effects of AGEs-mediated cardiovascular complication in T2DM involve various pathological changes such as plaque formation, arterial stiffening, and generalized endothelial dysfunction aided by prothrombotic gene expression [83-85]. These detrimental effects of AGEs can be explained at the cellular and extracellular level as shown in Figure 2.

AGEs-RAGE axis in Cardio-vascular complications

At cellular level AGEs mediate their effects through interaction with their receptors, especially receptor for advanced glycation end products (RAGE). The RAGE is recognized by multiple ligands and has been localized on endothelial cells, vascular smooth muscle cells, immune cells and many others [86]. The presences of RAGE on multiple cells indicate its involvement in pathways affecting the vascular system in diabetes [87]. AGE-RAGE interaction activates signaling cascades leading to enhance production of reactive oxygen species (ROS), oxidative stress , inflammation, adhesion molecule expression, endothelin-1, plasminogen activator inhibitor 1 (PAI-1), TNF-α, chemoattraction of inflammatory cells, smooth muscle and fibroblast proliferation, autophagy, and apoptosis [88-90]. AGE-RAGE interaction modulates the cellular properties that possibly promote pro-inflammatory and pro-coagulant gene pathways

through stimulation of signaling molecules such as ERK1/2, p21RAS, MAP kinases, NF-κB, cdc42/rac, and JAK/Stat and adversely affect the cardiovascular health in diabetes [91, 92]. Cipollone *et al*, have studied the association of AGE-RAGE interaction and RAGE over-expression in human diabetic plaque macrophages by an increased inflammatory reaction, cyclooxygenase-2/prostaglandin E synthase-1 expression that may contribute to plaque destabilization through induction of metalloproteinase expression [93]. Also, AGE-RAGE system activates inflammation in plaques and atheromas. Therefore, therapeutic approaches are now targeting the AGE-RAGE system to prevent the development of atherosclerosis [94].

Glycation of cellular and extra-cellular components in diabetic-CVD

AGEs are also involved in the covalent modifications and cross-linking of serum and extracellular matrix (ECM) proteins, lipids and nucleic acid leading to perturbation of their structure and functions. Proteins of ECM have slow turnover rate and longer half-life which make them more prone to glycation reaction and cross-linking under hyperglycemic conditions. Modification of ECM proteins and cross-linking interferes with cell-matrix and matrix-matrix interactions, leading to profibrotic action, decreased elasticity, increased stiffness and narrowing of vessels and other hallmarks of atherosclerosis [14, 95]. Cellular proteins also undergo the non-enzymatic glycation reaction by glucose and its derivatives like glucose-6-phosdphate, glyceraldehyde-3-phosphate, dihydroxyacetone-phosphate, GO, and MGO. Cellular AGEs have also been known to activate signaling pathways further impacting the diabetic vascular complications [96]. AGEs also induce cross-linking with intracellular proteins that participate in Ca^{2+} homeostasis resulting in cardiomyocyte dysfunction [97]. AGE-RAGE interaction is also found to be associated with decreased Ca^{2+} levels by up-regulated ryanodine receptor which is involved in maintaining ionic balance during systolic and diastolic phases [98].

Development of cardiovascular complications in T2DM is also associated with increased incidence of low-density lipoprotein (LDL) oxidation, glycation of

paraoxonase (PON1), and high-density lipoproteins (HDL) [99]. Oxidation of LDL in arterial wall is the prime step in initiation and progression of atherosclerosis by foam cell formation. Recent studies have reported that glycated LDL can evade recognition by LDL receptors and can approach to arterial wall [100]. Non-enzymatic glycation of LDL is also responsible for impairment of hepatic receptor-mediated uptake and its removal. As a result, AGEs-modified-LDL is trapped in sub-endothelium, causing its retention in the aortic wall where they are internalized by macrophages resulting in foam cell formation [101-103]. Glycation of LDL also makes them more vulnerable to cross-linking with collagen in the arterial wall. Elevated lipid-linked AGEs levels in LDL have also been noticed in T2DM patients [104]. Glycation of HDL also influences inflammation and affects the removal of cholesterol leading to the development of atherosclerosis [105]. Paraoxonase is an HDL-associated associated enzyme having anti-atherogenic properties and protects the LDL and cell membranes from oxidation. Glycation of PON1 is found to decrease its activity in diabetes mellitus leading to the development of premature atherosclerosis [17, 106, 107].

AGEs and Oxidative Stress in diabetic-CVD

Diabetic patients are exposed to high oxidative stress, increased reactive species (RS) generation, and decreased anti-oxidant defense mechanism. Hyperglycemia-induced ROS generation unveils the pathophysiology of CVD in diabetes and increased production of ROS triggers the inflammatory cascades responsible for the pathogenesis of cardiovascular complications [108, 109]. Level of transcription factors such as TNF-α and NF-κB is modulated by increased RS production mediated signal transduction pathways enhancing the proinflammatory events including inflammatory adhesion molecules, IL6, IL1, and cytokines [110-112]. The AGE-RAGE interaction is also involved in increased RS generation through stimulation of certain signaling mediators like extracellular-signal-regulated-kinase (ERK), phospholipase A2, phophoinositide3-kinase activation, activation of NADPH oxidase, iNOS, protein kinase C (PKC) and p38 mitogen-activated protein kinase (MAPK) [113-115]. Increased ROS production by

mitochondria also triggers the inflammatory cascades in diabetes and prolonged exposure to high levels of ROS leads to oxidation, peroxidation and glyoxidation reactions resulting in increased oxidative stress markers such as protein carbonyl, oxidation of thiol group, lipid peroxidation, advanced oxidation protein products, and 8-OHdG [17, 116]. Oxidative injury to biomolecules has also been observed in tissues and blood of diabetics with high AGE concentrations [117, 118]. *In vitro* and *in vivo* studies have reported that increased ROS production by AGE-RAGE interaction causes DNA damage that induces endothelial cell death by triggering the apoptotic pathway [119, 120].

AGEs and Endothelial Cell Dysfunction

Endothelial dysfunction is the hallmark for the development of cardiovascular complications in T2DM. Presence of RAGE on endothelial cell surface suggests its relevance in endothelial dysfunction by interacting with AGEs in T2DM. , Lowered nitric oxide (NO) production, increased ROS generation, enhanced expression of adhesion molecules, chemokine and cytokine are the hallmarks of endothelial dysfunction [121]. These conditions lead to inflammation, vasoconstriction, oxidative stress, myofibroblast migration, and proliferation inside the endothelial layer of vessels, all of which play a vital role in the development and progression of vascular complications in T2DM [122]. Under hyperglycemic condition endothelial cell proteins such as fibroblast growth factor and mitochondrial proteins undergo non-enzymatic glycation reactions affecting the vascular properties of cells by increased superoxide production, altering mitogenic and eNOS activity [123, 124].

Serum level of AGEs is negatively associated with the extent of endothelium-dependent vasodilation in T2DM patients [125]. Nitric oxide acts as an anti-atherogenic factor due to its effective vasodilatory, anti-inflammatory, and anti-proliferative influence [110, 126]. Increased ROS production by AGEs is one of the reasons for inactivation of NO as well their conversion to peroxynitrite form and thereby, affecting the integrity of endothelial cells. Formation and accumulation of AGEs inside the endothelial cells is also found to be associated with reduced eNOS gene expression and increased eNOS-mRNA

degradation [126]. AGE-RAGE interaction on endothelial cells also results in enhanced production of asymmetric dimethylarginine (ADMA) which is an endogenous inhibitor of eNOS and is one of the strongest marker of cardiovascular disease progression [127]. AGEs are also involved in NO quenching and inactivation of endothelium-derived NO [88]. Uhlmann *et al*, have reported a significant reduction in NO production in AGEs-treated cells *in vitro*. Their results implicated that AGEs have a role in the modulation of NO activity in diabetic pathophysiology [128]. Ren *et al*, have demonstrated the involvement of AGEs in reducing eNOS expression and NO bio-availability by increasing the oxidative stress development through activation of p38 and ERK1/2 in human coronary artery endothelial cells in vitro [29]. Therefore, accumulation of AGEs and the AGE-RAGE interaction has an important impact on the pathogenesis of diabetic-associated CVD by affecting the vasodilating properties of endothelial cells. AGE-RAGE axis also provokes the expression of p22hox and gp91hox, which are reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in endothelial cells and causes its dysfunction [28].

Involvement of AGEs has also been noticed in the production of vascular endothelial growth factor (VEGF) by endothelial cells and thereby, involved in atheroma formation. The activation of NF-κB by AGEs increases the secretion of VEGF that prevent the repair of endothelial lesions resulting in atherogenesis, stimulates the differentiation of monocyte to macrophages and the accumulation of oxidized LDL in the vasculature leading to foam cell formation [29, 129]. In addition, AGE-RAGE involvement has also been observed to inhibit the prostacyclin production and generation of PAI-1 in endothelial cells [130]. Formation and accumulation of AGEs have also been implicated in platelet activation and aggregation, stimulation of pro-coagulant activity, thrombus formation and endothelial cell damage mediated by up-regulation of protease-activated receptor-1and-2 potentiates thrombin [131, 132]. Decreased endothelial progenitor cell (EPC) function and mobilization poses a major risk for developing cardiovascular complications in T2DM [133]. The AGEs-RAGE interaction is found to augment the apoptotic pathways and suppress the migration and tube formation of late EPC by

down-regulation of Akt and cyclooxygenase-2 [134]. Moreover, glycation of Arg-Gly-Asp motif of fibronectin by AGEs results in impairment of vascular repair by inhibiting EPC adhesion, migration, and spreading [134].

Vascular complications are also characterized by the adhesion and transmigration of monocyte into the sub-endothelial space. The AGE-RAGE interactions are found to enhance this process by activation of pro-inflammatory molecules such as NF- κB that causes the overexpression of proinflammatory genes and adhesion proteins that aids monocyte adhesion to endothelial cells [103, 135, 136]. Foam cells and fatty streak formation take place in the vessel wall by monocyte and oxidized lipid at the adhesion site. These fatty streaks mature into advanced lesions with a fibrous cap that can dislodged resulting in an infarct or a stroke [137]. These observations suggest that, AGEs have a definitive role in development and progression of vascular injuries observed in diabetes.

AGEs and Vascular Smooth Muscle Cell Modifications

Recently researchers have identified the phenotype transformation of vascular smooth muscle cell (VSMC) into macrophages during cardiovascular pathology [138]. *In vitro* studies have shown the effects of AGEs on increased proliferative activity and production of fibronectin in cultured smooth muscle cells (SMC). Transforming growth factor-β might act as a mediator in AGE-induced fibronectin production in SMC through AGE-RAGE interactions [139]. *In vivo*, the effect of AGEs on the growth of SMC has also been noticed and is mediated by increased production of cytokines or growth factors [140]. Expansion of neointima is a unifying feature of atherosclerosis. Significant decreased in neointimal expansion, SMC proliferation, migration, and expression of extracellular matrix proteins have been demonstrated in homozygous RAGE-null mice. Taken together these data highlight the involvement of AGE-RAGE axis in modulating the SMC properties and suggesting an important pharmaceutical target for suppression of neointima expansion [44, 140]. Vascular smooth muscle cells phenotype transformation and calcification is one of the main pathological manifestations of atherosclerosis [141].

Recently Bao *et al*, have shown the effect of AGEs on VSMC derived foam cell formation and phenotype transformation. In their study, they identified the effect of CML on decreased expression of VSMC markers and increased expression of macrophage markers. They also noticed the involvement of AGEs in SMC migration and the secretion of pro-inflammatory factors [142]. Another recent study by Xing *et al*, explained the associated mechanism of phenotype transformation of VSMC to macrophages by AGEs during atherosclerosis. They noticed AGEs induced activation of RAGE/TLR4/FOXC2 signaling in macrophages with high expression of delta-like ligand4 (DII4) during M1 polarization. These altered macrophages promoted phenotype conversion of VSMC through DII4/Notch pathway after cell-to-cell contact [143].

ANTI-AGE THERAPIES

The deleterious effects of AGEs in the development and progression of diabetic-vascular complications have driven the focus of pharmacological intervention towards attenuating the effects of AGEs. Although lifestyle modification, better glycemic control, regular physical activity, smoking cessation, restriction of AGE-rich diet have been reported to reduce the availability of precursors for glycation reactions and AGEs formation in T2DM [144-146]. A plethora of studies over the last few decades have been dedicated to in searching for pharmacological agents capable of interfering with glycation reactions and their sequalae. The underlying mechanism of action of these proposed drugs are based on AGEs inhibitors, AGE cross-link breakers, detoxifying the dicarbonyls intermediates and AGE-RAGE signaling blockers shown in Figure 3 [147, 148]. Not any AGE-modifiers have been approved as drugs as yet, although some AGE-associated medications are in clinical and preclinical testing. Phytochemicals having antioxidant and anti-inflammatory properties have the potential to arrest the detrimental effects of AGEs and downstream consequences of AGE-RAGE pathway [149].

Inhibition of Endogenous AGEs formation

First drug that was discovered to impede endogenous AGEs formation was aminoguanidine having an guanidine group that is capable of trapping α-dicarbonyl product of early glycation reactions and thereby, preventing the subsequent reactions with proteins [150, 151]. Bolten *et al*, have demonstrated the role of aminoguanidine in reducing proteinuria and progression to retinopathy, however due to its side effects, it is unlikely to be used for therapeutic purposes [152]. Compounds structurally related to aminoguanidine such as ALT-946 and OPB-9195 have been developed and tested as potential drugs. ALT-946 therapy was found to reduce the renal AGEs accumulation, and reduced albumin excretion in animal model [153]. OPB-9195 is an antagonist of peroxisome proliferators-activated receptor-γ and inhibits the glycoxidation and lipoxidation reactions. In animal models OPB-9195 decreased the progression of nephropathy, lowered the blood pressure, and the serum level of AGEs [154, 155]. LR-90 is another aromatic compound having anti-AGEs properties due to its metal chelating ability and its interaction with dicarbonyl compounds. It affords renoprotection such as improved renal albuminuria, reduction of connective tissue growth factors, fibronectin and collagen deposition in experimental model of type 1 and type 2 nephropathy [156]. The TM2002 is a powerful AGEs inhibitor having a transition metal chelating properties and is fairly non-toxic. It improves renal and cardiac lesion, decreased infarct volume in different animal models [157, 158]. Benfotiamine is a pro-drug of thiamine monophosphate having AGEs-lowering property, mediated through preventing dicarbonyl formation [159, 160]. In a pilot study, Brownlee *et al*, observed that treatment along with α-lipoic acid improved complications in type 1 and type 2 diabetic patients. Pyridoxamine has also been demonstrated to intervene the glycation process by blocking the transformation of amadori products into AGEs [161]. They have the ability to trap ROS, thereby blocking the oxidative degradation of amadori intermediates and preventing the formation of AGEs [162, 163].

Preformed AGE-breakers

Among the deleterious effects of AGEs accumulation cross-linking of ECM is of prominence that results in cardiovascular stiffness. Phenylthiazolium bromide was the first reported AGEs cross-link breaker that is not stable in aqueous solution [164]. Several of its derivates have now been derived such as ALT-711 or alageberium having the ability to break the AGEs cross-links. The precise mechanism of their action relies on reaction with carbonyl groups present in AGEs cross-links and cleavage of carbon-carbon bonds. Application of alageberium in animal models has proved to be effective in reducing large artery stiffness, blood vessel fibrosis, attenuating atherosclerosis, diabetic nephropathy, and hypertension [165, 166]. Recently the role of aptamers is being explored in biomedical and pharmaceutical industries [167]. Aptamers are a group of short and single-stranded DNA or RNA molecule having the ability to bind with high affinity/specificity to a variety of proteins. DNA aptamers raised against AGEs have been observed to bind and ameliorate AGE associated effects [168]. These specific DNA aptamers can become novel therapeutic agents for AGE-related pathologies.

AGE-RAGE signaling blockers/ RAGE antagonist

In vitro and in vivo studies have confirmed that AGE-RAGE axis is one of the major pathways for diabetic-vascular complications. Therefore, it would be an ideal target to prevent the development and progression of complication in T2DM. Pharmacological agents that focus the AGE-RAGE axis could function through different means such as inhibiting the RAGE expression, altering the AGE-RAGE signaling or by raising the blood level of sRAGE to trap AGEs. sRAGE are formed by either alternative gene splicing of RAGE gene or proteolytic cleavage of membranous RAGE. Administration of sRAGE has shown to decrease albuminurea, glomerulosclerosis and diabetic-CVD [169, 170]. Statin and thiazolindinediones have been shown to ameliorate RAGE expression in conjugation with increased sRAGE [171, 172]. The proposed underlying mechanism of statin and thiazolindinediones have also described by activation of peroxisome proliferators-activated receptor-γ, which could inhibit the phosphorylation of ERK1/2 and down regulate NF-κB, thereby lowering the expression of inflammatory cytokines

and RAGE [173, 174]. Other molecules such as glucagon-like peptide-1(GLP-1) and its analog exendin have also demonstrated to decrease RAGE expression through suppressing NF-κB and decreasing ROS production by inhibiting NADPH oxidase activity [175, 176]. Studies have also reported the involvement of GLP-1 and exendin in reducing the activation of AGE-RAGE axis and its associated complications such as atherosclerosis, diabetic cardiomyopathy etc [177, 178]. Recently, the involvement of RAGE inhibitors namely FPS-ZM1 and PF-04494700 has shown neuro-protective effects against ischemic brain injury in rat model and β-amyloid structures in clinical trials respectively for Alzheimer's disease [179, 180]. Effect of FPS-ZM1 as RAGE inhibitors is found to be associated with decreased inflammation and oxidative stress by targeting other ligands of RAGE such as S100, high-mobility group protein 1 (HMGB1), and amyloid β-protein [180-183]. Promising outcome of RAGE blockers such as FPS-ZM1 and PF-04494700 in neurodegenerative diseases provides the rationale to study their effects in T2DM patients against AGEs.

AGEs and Hypoglycemic drugs

The effects of many hypoglycemic drugs have also been studied in context of decreasing the AGEs level, ameliorating the effects of AGE-RAGE axis and showed positive results. Prasad *et al* have reported the effects of rosiglitazone in inhibiting the AGE-RAGE interaction and found elevated sRAGE levels [169]. Similar results have been reported in randomized placebo-controlled study of 111 patients with T2DM-CVD, where increased sRAGE and decreased inflammatory markers were reported after six months of rosiglitazone treatment [184]. Effect of glimepiride beyond glycemic control have been reported in reduction of toxic glyceraldehydes-derived AGEs levels and increased colony-stimulating factors to potentially repair tissue damage in T2DM patients[185]. Metformin treatment was found to inhibit development of adverse myocardial structural and functional changes by inhibiting the production and accumulation of AGEs [186, 187]. Metformin also inhibited the AGEs induced vascular smooth muscle proliferation [188]. Animal and *in vitro* models have shown the efficacy of

Dipeptidy peptidase-4 inhibitors such as sitagliptin, cilizytin, vildagliptin and linalgliptin in inhibiting the glycosylation process, down regulate the levels of AGEs, RAGE, oxidative stress markers, decreased expression of VCAM-1, PAI-1, and ICAM-1 [189-192]. Another hypoglycemic drug glucagon-like peptide analogue liraglutide was also found to ameliorate the atherogenesis by inhibiting AGEs-induced expression of RAGE in mice model [193].

CONCLUSION

Type 2 Diabetes Mellitus imposes both clinical and economic burdens on the health care system. Recent reports have confirmed that CVD represents a substantial burden on the treatment of T2DM at both patient and population level. The pathophysiology of hyperglycemia in T2DM is closely associated with AGEs formation, accumulation, and their deleterious effects. The adverse effects of AGEs accumulation include many important biochemical reactions that are central to the development and progression of cardio-vascular complications in T2DM. AGEs-mediated cardiovascular complications show many pathological changes such as plaque formation, arterial stiffening, neointimal proliferation, vasoconstriction, oxidation of LDL and endothelial dysfunction. The probable mechanisms through which AGEs exert their detrimental effects include increased ROS generation, oxidative stress development, decreased NO production and its inactivation, inflammation, adhesion molecules expression, cross-linking of proteins, and prothrombotic gene expression. AGE-RAGE interactions also alter the cellular properties by promoting pro-inflammatory and pro-coagulant pathways acting through modulation of signaling molecules such as ERK1/2, cdc42/rac, p21RAS, TNF-α, MAP kinases, NF-κB, and JAK/Stat that adversely affect the cardiovascular health in T2DM. AGE-ARGE axis is also involved in modulating the SMC properties and neointima expansion, where it mediates SMC proliferation, phenotype transformation of VSMC into macrophages during cardiovascular pathology. Therefore, clinical and experimental research is now focused on AGEs as new biomarkers or therapeutic target to prevent the development and progression of

diabetic vascular complications. Based on AGE-mediated effects in pathogenesis of T2DM and its complications, pharmacological approaches are exploring combination therapies targeting multiple pathways based on inhibitors of AGEs formation, AGE cross-link breakers, free radical scavengers, anti-inflammatory therapies, detoxifying the dicarbonyls intermediates and AGE-RAGE signaling blockers that may attenuate AGE-mediated effects in diabetic cardio-vasculature. The uses of phytochemicals having antioxidant and anti-inflammatory properties are promising strategies to arrest the detrimental effects of AGEs. Also, there is a need to develop more specific and sensitive methods for the assay of circulatory AGEs. An epidemic of diabetes over the past half-century has also been associated with increased consumption of modern heat-processed and highly palatable-AGE-rich diet. Therefore, lifestyle modification including dietary AGE restriction, regular exercise and cessation of smoking are some of the important interventions and practical ways to attenuate the effects of AGE-RAGE axis and AGE-associated pathways.

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