Role of Hyperglycemia-Induced Advanced Glycation End Product (AGE) Accumulation in Atherosclerosis

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There is a growing body of evidence that cumulative hyperglycemic exposure plays a central role in the development and progression of atherosclerotic cardiovascular disease in diabetic patients. Monosaccharides, such as glucose, fructose, and glyceraldehyde can react non-enzymatically with amino groups of proteins, lipids, nucleic acids to form senescent macromolecules termed advanced glycation end products (AGEs), whose formation and accumulation has been known to progress in diabetic patients, especially in those with a long history of disease. The sustained accumulation of AGEs could contribute to the phenomenon of metabolic memory or legacy effects observed in long-term follow-up clinical studies of diabetic patients. AGE modification alters the structural integrity and function of various types of macromolecules, and interaction of AGEs with a receptor for AGEs (RAGE) has been shown to evoke inflammatory and thrombotic reactions. Therefore, the AGE–RAGE axis is a novel therapeutic target of atherosclerotic cardiovascular disease in diabetic patients. In this paper, we briefly review the pathological role of AGEs and their receptor RAGE system in atherosclerotic cardiovascular disease, including peripheral artery disease and discuss the clinical utility of measuring AGEs in evaluating the severity of atherosclerosis in patients with diabetes.

Keywords: AGEs, atherosclerosis, RAGE, PAD

Introduction

Atherosclerotic cardiovascular disease (CVD), such as acute myocardial infarction, stroke, and peripheral artery disease (PAD) is a highly prevalent complication of diabetes, and is also a leading cause of death in diabetic patients. More than 50% of diabetic patients have been shown to die from CVD in industrialized countries, and the risk of limb amputation due to PAD is increased dramatically in patients with diabetes. Furthermore, there is accumulating evidence that, in the development and progression of atherosclerotic CVD, including PAD in diabetes, cumulative hyperglycemic exposure plays a central role. Indeed, in contrast to the previous report of Haffner et al., two clinical studies have shown that the risk of CVD in newly identified diabetic subjects or patients with a relatively short history of diabetes is not equivalent to non-diabetic individuals with a previous history of CVD, while patients with a more than 8 years history of diabetes had a comparable risk. In addition, the association of diabetes with the increased risk of CVD death was significantly attenuated after adjusting for HbA1c, but not lipid parameters, body mass index, inflammatory biomarkers, or systolic blood pressure.

Monosaccharides, such as glucose, fructose, and glyceraldehyde can react non-enzymatically react with amino groups of proteins, lipids, nucleic acids to form senescent macromolecules termed advanced glycation end products (AGEs), whose formation and accumulation has been known to progress in diabetic patients, especially in those with a long history of disease. Since AGE-modified macromolecules are hardly metabolized and eliminated from the body, formation and accumulation of AGEs could reflect cumulative hyperglycemic exposure in patients with diabetes. Moreover, AGEs increase oxidative stress generation in various kinds of cells through the interaction with a cell surface receptor, receptor for AGEs (RAGE) and resultantly induce RAGE expression. Therefore, AGEs could cause the sustained activation of RAGE-signaling pathways and further stimulate the topical formation and accumulation of AGEs, thus, forming...
a positive feedback loop, which may also account for the phenomenon of metabolic memory or legacy effects observed in long-term follow-up clinical studies of diabetic patients.\textsuperscript{13-25} These observations suggest that the AGE–RAGE axis is a novel therapeutic target of atherosclerotic CVD in patients with diabetes. Therefore, in this paper, we briefly review the pathological role of AGEs and their receptor RAGE system in atherosclerotic CVD, including PAD and discuss the clinical utility of measuring AGEs in evaluating the severity of atherosclerosis in patients with diabetes.

Role of AGEs and RAGE Axis in Atherosclerosis

The modification of AGE alters the structural integrity and function of various types of macromolecules, and the interaction of AGEs with RAGE evokes oxidative stress, inflammatory, thrombotic and fibrotic reactions in numerous kinds of cells, thereby, becoming involved in atherosclerotic CVD.\textsuperscript{13-25}

Endothelial dysfunction and vascular inflammation

Endothelial cell-derived nitric oxide (NO) plays a protective role against atherosclerosis; not only does it stimulate vasodilation, but it also inhibits inflammatory reactions, platelet activation and aggregation.\textsuperscript{26-35} Impaired endothelial cell-derived NO synthesis and/or bioavailability could contribute to endothelial dysfunction, an early characteristic feature of atherosclerosis, which could predict future cardiovascular events in high-risk patients such as diabetic subjects.\textsuperscript{26-35}

AGEs have been shown to inhibit endothelial cell-derived NO production via the suppression of endothelial NO synthase expression.\textsuperscript{35-37} In addition, the AGE–RAGE-induced oxidative stress generation could activate NO, resulting in the increased formation of toxic byproduct of NO, peroxynitrite.\textsuperscript{35-37} Furthermore, the interaction of AGEs with RAGE stimulates the generation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase in endothelial cells, mesangial cells, and renal proximal tubular cells.\textsuperscript{38-41} The circulating levels of AGEs have been associated with ADMA and/or endothelial dysfunction in high-risk patients for CVD, including end-stage renal failure patients with diabetes.\textsuperscript{40,41}

Accumulating evidence has suggested that atherosclerosis is intrinsically an inflammatory disease.\textsuperscript{42-49} Vascular inflammation could also play a role in endothelial dysfunction, and circulating levels of high-sensitivity C-reactive protein, an inflammatory biomarker have been shown to predict future adverse cardiovascular events in humans independent of conventional risk factors.\textsuperscript{42-49}

The engagement of RAGE with AGEs evokes oxidative stress and inflammatory reactions in vascular wall cells and, subsequently, elicits the activation of NF-κB, which could promote vascular inflammation.\textsuperscript{13-25} We have found that the serum levels of AGEs are correlated with inflammatory biomarkers such as monocyte chemoattractant protein-1 and soluble form of vascular cell adhesion molecule-1 in type 2 diabetic patients and independently associated with vascular inflammation evaluated by \textsuperscript{18}F-fluorodeoxyglucose–positron emission tomography.\textsuperscript{50-52}

The tissue accumulation levels of AGEs can be evaluated non-invasively by skin autofluorescence (SAF) over the entire 420–600 nm emission spectrum to that over 300–420 nm with a desktop computer.\textsuperscript{53-55} SAF was inversely associated with anti-oxidative capacity of high-density lipoprotein in patients with type 2 diabetes, and were independently correlated with high-sensitivity C-reactive protein levels in patients on hemodialysis. These observations suggest further the pathological role of AGE accumulation in oxidative stress and inflammatory reactions in diabetes.\textsuperscript{56,57}

Arterial stiffness

Cross-linking by AGEs of extracellular matrix proteins such as collagens and elastin has been shown to contribute to arterial stiffness, a predictor of future cardiovascular events in both apparent healthy subjects and high-risk patients for CVD.\textsuperscript{58,59} Furthermore, SAF was independently correlated with aortic pulse wave velocity, a marker of arterial stiffness in type 1 diabetic patients without a history of CVD.\textsuperscript{60}

Plaque formation and angiogenesis

AGE modification of apolipoprotein B100 makes low-density lipoprotein more atherogenic.\textsuperscript{61,62} Moreover, the AGE–RAGE-induced oxidative stress has been shown to decrease the expression levels of adenosine triphosphate-binding membrane cassette transporter A1 (ABCA1) and ABCG1 in cultured macrophages and resultantly suppress cholesterol efflux from macrophages to apolipoprotein A1 and high-density lipoprotein, respectively.\textsuperscript{63} These observations suggest that the activation of the AGE–RAGE axis not only promotes the atherosclerotic plaque formation, but also impairs the reverse cholesterol transport, thereby, being involved in accelerated atherosclerosis in diabetes.\textsuperscript{64}

We have found previously that AGEs induce pathological angiogenesis by stimulating the autocrine production of vascular endothelial growth factor through the interaction of RAGE.\textsuperscript{65-67} Plaque angiogenesis could function as conduits for the entry of inflammatory cells into the atherosclerotic lesions, and are associated with plaque instability such as plaque rupture and intraplaque hemorrhage.\textsuperscript{68-70} Furthermore, recently, SAF has been shown to
be associated with plaque vulnerability assessed by optical coherence tomography in patients with CVD.71) These findings suggest the active contribution of AGE–RAGE axis to atherosclerotic plaque instability in diabetes.

**Platelet activation, thrombosis, and hypercoagulability**

The interaction of AGEs with RAGE inhibits an antithrombotic prostanoid, prostacyclin production by endothelial cells, while it stimulates plasminogen activator inhibitor-1 generation and activity, thereby, stabilizing the arterial thrombi.72) In addition, AGEs have been shown to induce platelet activation and aggregation and enhance the coagulation cascade, which in concert could play a crucial role in the development of atherothrombotic CVD in diabetes.73–78)

**Impaired endothelial cell repair**

By inducing the apoptotic cell death of endothelial progenitor cells (EPCs) and simultaneously suppressing their migration and tube formation in vitro, the AGE–RAGE interaction could impair endothelial cell repair.79) Moreover, we have found previously that the serum levels of AGEs are inversely associated with the number and migratory activity of EPCs in healthy volunteers.80) Given that the reduced number and migratory activity of EPCs could be a predictor of future cardiovascular events, the AGE–RAGE axis may contribute to the increased risk of CVD partly via the impairment of endothelial cell repair.81) SAF has been reported to be independently associated with the decreased number of circulating EPCs in end-stage renal disease patients.82)

**AGEs as a Biomarker of CVD**

SAF was significantly higher in the PAD patients than the controls, especially in the PAD patients with cardiovascular comorbidity.83) Furthermore, SAF has been shown to be an independent predictor of amputation due to critical limb ischemia and is also associated with 5-year mortality and fetal and non-fetal major cardiovascular events in patients with PAD.84)

Circulating levels of pentosidine, one of the well characterized AGEs, were significantly higher in diabetic patients with PAD compared with non-diabetic individuals, and were inversely associated with ankle–brachial index.85) Moreover, pentosidine and carboxymethyllysine levels were correlated with the severity of PAD and independently associated with the presence of critical limb ischemia.86) SAF was elevated in patients with ST-elevation myocardial infarction and could be a predictor of future major adverse cardiac events in these patients.87) SAF was also correlated with cumulative diabetic exposure and cardiac mortality in patients with diabetes.88) The circulating levels of AGEs have been reported to predict total and cardiovascular disease mortality in both type 1 and type 2 diabetic patients.89,90)

**Conclusion**

Here, we briefly reviewed the role of AGE–RAGE axis in the development and progression of atherosclerotic CVD, including PAD. The inhibition of the AGE–RAGE axis may be a therapeutic target for CVD in diabetes.

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**Author Contributions**

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