Molecular Medicine

Advanced Glycation End Products: A Molecular Target for Vascular Complications in Diabetes

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A nonenzymatic reaction between reducing sugars and amino groups of proteins, lipids and nucleic acids contributes to the aging of macromolecules and subsequently alters their structural integrity and function. This process has been known to progress at an accelerated rate under hyperglycemic and/or oxidative stress conditions. Over a course of days to weeks, early glycation products undergo further reactions such as rearrangements and dehydration to become irreversibly cross-linked, fluorescent and senescent macroprotein derivatives termed advanced glycation end products (AGEs). There is a growing body of evidence indicating that interaction of AGEs with their receptor (RAGE) elicits oxidative stress generation and as a result evokes proliferative, inflammatory, thrombotic and fibrotic reactions in a variety of cells. This evidence supports AGEs' involvement in diabetes- and aging-associated disorders such as diabetic vascular complications, cancer, Alzheimer's disease and osteoporosis. Therefore, inhibition of AGE formation could be a novel molecular target for organ protection in diabetes. This report summarizes the pathophysiological role of AGEs in vascular complications in diabetes and discusses the potential clinical utility of measurement of serum levels of AGEs for evaluating organ damage in diabetes.

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INTRODUCTION

Diabetes is a global health challenge. In the recent report in the *IDF Diabetes Atlas*, the prevalence and number of diabetes cases in the world are estimated to be 8.3% and 387 million, and one person dies from diabetes every 7 s (1). Although diabetic vascular complications, including coronary heart disease and apoplexy, could mainly account for disabilities and high mortality rate in patients with diabetes (1,2), the risk of other aging-associated disorders such as cancer, Alzheimer's disease and osteoporosis is also increased in diabetic subjects (3–6). As a result, average life span is reduced by more than 20 years in middle-aged people with type 1 diabetes and by up to 10 years in middle-aged, type 2 diabetic patients compared with nondiabetic subjects (7–9). The recent report of Emerging Risk Factors Collaboration showed that 40-year-old diabetic subjects without known cardiovascular disease (CVD) at the time of enrollment died about 6.3 years earlier than nondiabetic subjects (3). Furthermore, cumulative hyperglycemic exposure and subsequently enhanced accumulation of advanced glycation end products (AGEs) have been shown to contribute to the development and progression of these diabetes- and aging-related disorders (2,4–6). Therefore, inhibition of AGE formation could be a novel molecular target for various devastating and life-threatening disorders. In this report, we review the progress of research on AGEs from 1994 to the present, especially focusing on vascular complications in diabetes, and we discuss how our studies in the field of AGEs have been influenced by and contributed to the article published in *Molecular Medicine* in 2001 (10). Moreover, we would like to refer to how the information in the article may alter the diagnosis and treatment of diabetic vascular complications now or in the future.

METABOLIC MEMORY

The Diabetes Control and Complications Trial–Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) research has revealed that beneficial effects of intensive therapy on microvascular complications in type 1 diabetic patients persist for 14–18 years after the DCCT, despite deterioration of blood glucose control (11–13). Furthermore, intensive glycemic control during

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the DCCT resulted in decreased progression of intima-media thickness and subsequently reduced the risk of nonfatal myocardial infarction, stroke or death from CVD by 57% 11 years after the end of the trial (14–16). Recently, original intensive therapy for 6.5 years has been shown to yield benefits on all-cause mortality rate after a mean 27 years of follow-up in patients with type 1 diabetes (17). In addition, a follow-up study of the U.K. Prospective Diabetes Study (UKPDS), called UKPDS80, has also shown that benefits of an intensive therapy in patients with type 2 diabetes are sustained after the cessation of the trial (18). In this study, despite an early loss of glycemic differences between the original intensive therapy group and the conventional one, a continued reduction in microvascular risk and emergent risk reductions for myocardial infarction and death from any cause were observed during 10 years of post-trial follow-up (18). These findings demonstrate that so-called "metabolic memory" may cause chronic abnormalities in diabetic vessels that are not easily reversed, even by subsequent, relatively good blood glucose control, thus suggesting a long-term beneficial influence of early metabolic control (that is, the legacy effect) on the risk of diabetic vascular complications and death in both type 1 and type 2 diabetic patients.

AGEs AND RECEPTOR TO AGEs (RAGE)

AGEs are formed by the Maillard process, a nonenzymatic reaction between reducing sugars and the amino groups of proteins, lipids and nucleic acids that contribute to the aging of macromolecules (2,10,19). Under hyperglycemic and/or oxidative stress conditions, this process begins with the conversion of reversible Schiff base adducts to more stable, covalently bound Amadori rearrangement products (2,10,19). Over the course of days to weeks, these Amadori products undergo further rearrangement reactions to form the irreversibly cross-linked, fluorescent macroprotein derivatives, termed AGEs. About 10% of Amadori products could move to the irreversible process (20). AGEs are slowly degraded and remain for a long time in diabetic vessels, even after glycemic control has been improved (21,22).

Several types of AGE binding proteins have been reported (23). Among them, the receptor to AGEs (RAGE) is a cell surface receptor that belongs to the immunoglobulin superfamily and is a signal-transducing receptor for AGEs (23–27). There is a growing body of evidence that engagement of RAGE with AGEs elicits oxidative stress generation and results in evoking inflammatory and thrombogenic reactions in a variety of cells, thereby being involved in vascular complications in diabetes. Furthermore, AGEs are known to upregulate RAGE expression and induce sustained activation of nuclear factor-κB (NF-κB) (23–27). Therefore, it is conceivable that the AGE–RAGE–induced oxidative stress generation further potentiates the formation and accumulation of AGEs and subsequent RAGE overexpression. These positive feedback loops between AGEs and RAGE-downstream pathways could make a vicious cycle, thus providing a mechanistic basis for understanding why the phenomenon of metabolic memory exists in vascular complications in diabetes. Therefore, the biochemical nature and mode of action of AGEs are most compatible with the concept of metabolic memory (28,29).

PATHOPHYSIOLOGICAL ROLE OF AGEs IN VASCULAR COMPLICATIONS IN DIABETES

CVD

Vascular stiffness and inflammation. Cross-linking of proteins by AGE modification not only leads to an increase in vascular and myocardial stiffness, but also deteriorates structural integrity and physiological function of multiple organ systems, thus being involved in isolated systolic hypertension and diastolic heart failure (30).

There is a growing body of evidence, ranging from *in vitro* experiments to pathologic analysis and epidemiologic studies suggesting that atherosclerosis is intrinsically an inflammatory disease (31,32). Activation of the AGE–RAGE axis results in generation of intracellular oxidative stress generation and subsequent activation of NF-κB in vascular wall cells, which could promote a variety of atherosclerosis/inflammation-related gene expression, thereby contributing to the development and progression of CVD in diabetes (2,23–27).

Nitric oxide (NO) is the most potent endogenous vasodilator and, by the role of its antiinflammatory, antiproliferation and antithrombotic effects, it is widely recognized as an endogenous antiatherogenic factor (33–38). We, along with others, have shown that AGEs not only inhibit endothelial NO synthase expression in endothelial cells, but they also stimulate generation of peroxynitrite, a reactive intermediate and toxic product of NO with superoxide anion (33–35). Furthermore, the AGE–RAGE interaction enhances the production of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase in endothelial cells, mesangial cells and renal proximal tubular cells (35–38). Because ADMA is now considered one of the strongest biomarkers of CVD and chronic kidney disease progression (35), decreased production and/or impaired bioavailability of NO by the AGE–RAGE axis could also be involved in cardiorenal syndrome in diabetes.

Foam cell formation within the atherosclerosis. AGE modification impairs plasma clearance of low-density lipoprotein and transforms the lipoprotein into a more atherogenic and redox-sensitive mitogen-activated kinase stimulant in diabetic patients (39,40). Furthermore, we demonstrated that AGEs reduce adenosine triphosphate–binding membrane cassette transporter A1 (ABCA1) and ABCG1 levels in THP-1 cells and inhibit cholesterol efflux from THP-1 macrophages to apolipoprotein (apo) AI and HDL cholesterol, respectively (41). These findings suggest the involvement of the AGE–RAGE axis in impaired re-

verse cholesterol transport in diabetes and accelerated foam cell formation within the atherosclerotic lesions (41,42).

Thrombogenesis. AGEs not only cause platelet activation and aggregation, but also stimulate procoagulant activity by increasing expression of tissue factor, the main initiator of the coagulation cascade, which is responsible for thrombus formation (43). Furthermore, we recently found that AGEs could potentiate thrombin or factor Xa–evoked endothelial and renal cell damages via upregulation of protease-activated receptor-1 and -2 (44–46). These observations suggest that blockade of the crosstalk between AGE–RAGE axis and coagulation system by factor Xa inhibitors might be a novel therapeutic target for thromboembolic disorders in diabetes. In addition, AGEs inhibit prostacyclin production and induce plasminogen activator inhibitor-1 generation in endothelial cells through an interaction with RAGE (47). Therefore, AGEs may have the ability to cause platelet aggregation and fibrin stabilization, resulting in a predisposition to thrombogenesis, thereby contributing to the promotion of vascular injury in diabetes.

Pathological angiogenesis within the atherosclerosis. Plaque neovascularization is comprised of a network of capillaries that arise from adventitial vasa vasorum and extend into the intimal layer of atherosclerotic lesions (48–50). They are often found in areas rich in inflammatory cells, such as macrophages and T cells, and have been considered to function as conduits for the entry of leukocytes and nutrients into the artery wall. Moreover, plaque vessels are associated with plaque rupture, intraplaque hemorrhage and unstable angina (48,50). Therefore, ischemia and hypoxia due to microthrombus formation within the atherosclerotic plaques may further stimulate vascular endothelial growth factor (VEGF) expression and trigger pathological angiogenesis in these vulnerable lesions (51,52). Therefore, the AGE-induced pathological angiogenesis may contribute to plaque growth and instability within the atherosclerotic plaques in diabetes.

Impaired endothelial cell repair. Diabetes is associated with endothelial dysfunction and decreased endothelial progenitor cell (EPC) function and mobilization, which could contribute to accelerated atherosclerosis and increased risk for CVD in diabetic patients (53). AGEs enhance apoptosis and suppress migration and tube formation of late EPCs through the interaction with RAGE via downregulation of Akt and cycloxygenase-2 (54). AGEs have also been shown to cause a reduction of length growth and EPC incorporation into the sprouts in association with RAGE overexpression and p38 mitogen-activated protein kinase activation (55). Moreover, AGE modification of vascular substrates impair vascular repair by inhibiting EPC adhesion, spreading and migration via glycation of the Arg-Gly-Asp motif of fibronectin (56).

Vascular calcification. AGEs have the ability to induce the osteoblatic differentiation of pericytes, thus contributing to the development of vascular calcification in atherosclerosis (57). Pericytes have the plasticity to differentiate into other mesenchymal cell types under various circumstances and may function as resting stem cells to be converted into smooth muscle cells, macrophage-like phagocytes or osteoblasts (48). Furthermore, activation of RAGE not only inhibits myocardin-dependent smooth muscle cell (SMC) gene expression, but also induces osteogenic differentiation of vascular SMCs through Notch/Msx2 induction, thus being involved in vascular calcification as well (58).

SMC proliferation. AGEs elicit reactive oxygen species generation and subsequently induce SMC proliferation through the interaction with RAGE via NADPH oxidase activation (59,60). AGE–RAGE–induced extracellular signal-related kinase activation is reported to increase Na^+/H^+ exchanger-1 activity, which leads to a decrease in intracellular H^+ and subsequently promotes a cell-cycle progression and SMC proliferation (61).

Diabetic Retinopathy

Pericytes are elongated cells of the mesodermal origin, wrapping around and along endothelial cells of small vessels (48). Because pericytes have played an important role in the maintenance of microvascular homeostasis, AGE-caused pericyte apoptosis could predispose the vessels to angiogenesis, thrombogenesis and endothelial cell injury, thus leading to full-blown clinical expression of diabetic retinopathy (2). Moreover, AGEs directly stimulate growth and tube formation of microvascular endothelial cells, the key steps of angiogenesis, through the interaction with RAGE by inducing VEGF expression (49).

AGEs have been shown to increase leukocyte adhesion to cultured retinal microvascular endothelial cells by inducing intracellular cell adhesion molecule-1 expression as well (62). This phenomenon is also apparent in nondiabetic mice infused with preformed AGEs, which results in significant leukostasis and blood-retinal barrier dysfunction in these mice (62). Because retinal VEGF induces intracellular cell adhesion molecule-1 expression, which could lead to leukostasis and breakdown of the blood-retinal barrier *in vivo* (63–65), the AGE-elicited proinflammatory reactions could be modulated by the blockage of VEGF.

Diabetic Nephropathy

Accumulation of AGEs in the kidney may contribute to the progressive alteration in renal architecture and loss of renal function in patients and rodents via various mechanisms, including their cross-linking properties of matrix proteins and activation of the downstream signaling (2,66,67). AGE formation on extracellular matrix proteins alters both matrix–matrix and cell–matrix interactions, and is involved in diabetic glomerulosclerosis. AGEs induce apoptotic cell death and VEGF expression in human cultured mesangial cells, as is the case in pericytes, a counterpart of mesangial cells in retinas (68). Because mesangial cells occupy a central anatomical position in the glomerulus and play a crucial role in

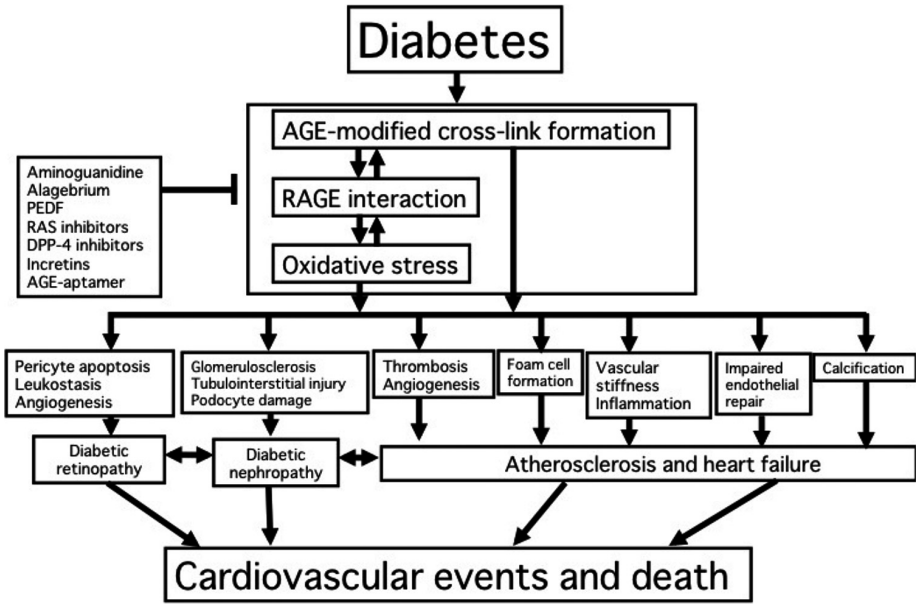

Figure 1. Pathophysiological role of AGEs in diabetic vascular complications.

maintaining structure and function of glomerular capillary tufts, the AGE-induced mesangial apoptosis and dysfunction may contribute in part to glomerular hyperfiltration, an early renal dysfunction in diabetes. Podocyte loss is a common and early feature in human and experimental diabetic nephropathy (69). AGEs have also been shown to induce podocyte damage and detachment (70). Furthermore, AGEs induce transforming growth factor-β expression in renal constituents such as podocytes, mesangial cells and proximal tubular cells, thereby contributing to glomerulosclerosis and tubulointerstitial fibrosis in diabetic nephropathy as well (71,72). We posit a scheme to summarize the pathophysiological role of AGEs in vascular complications in diabetes (73) (Figure 1).

RAGE TRANSGENIC AND KNOCKOUT MICE

Diabetic $RAGE^{-/-}/apoE^{-/-}$ mice had significantly reduced atherosclerotic plaque area, which is associated with attenuation of leukocyte recruitment, decreased expression of proinflammatory mediators, reduced oxidative stress and AGE accumulation (74).

RAGE-overexpressing diabetic mice have exhibited progressive glomerulosclerosis with renal dysfunction, compared with diabetic littermates lacking the RAGE transgene (75). Furthermore, Wendt *et al.* (76) reported that diabetic homozygous RAGE null mice failed to develop mesangial matrix expansion or thickening of the glomerular basement membrane. They also claimed in their report that activation of RAGE in podocytes could contribute to expression of VEGF and enhanced attraction/activation of inflammatory cells in the diabetic glomeruli, causing albuminuria and glomerulosclerosis in diabetes (76). In addition, *db/db* or streptozotocin-induced diabetic mice have developed renal changes seen in human diabetic nephropathy such as glomerular hypertrophy, glomerular basement membrane thickening, mesangial matrix expansion, connective tissue growth factor overexpression and NF-κB activation, all of which are blocked by the administration of neutralizing antibody raised against RAGE (77,78).

Blood-retinal barrier breakdown and retinal leukostasis were significantly augmented in RAGE-transgenic diabetic mice, which were blocked by the systemic administration of a soluble form of RAGE (79). Inflammatory reactions and the AGE–RAGE–NF-κB pathway were enhanced in peripheral nerves of diabetic mice, all of which were prevented by RAGE gene deficiency or the addition of a soluble form of RAGE, which was associated with dramatic improvement in the loss of pain perception (80).

THERAPEUTIC INTERVENTIONS OF THE AGE–RAGE AXIS

Inhibitors of AGE Formation and Breaker

Various types of inhibitors of AGE formation or AGE breaker alagebrium have been shown to prevent vascular damage in diabetic animals (81,82). Indeed, aminoguanidine was reported to increase vascular elasticity, improve left ventricular arterial coupling and decrease vascular permeability in diabetic rats (82,83). Aminoguanidine prevented the decreased myocardial compliance and cardiac hypertrophy in diabetic animals (83). Furthermore, aminoguanidine treatment decreased accumulation levels of AGEs and reduced atherosclerotic plaque area in the thoracic and abdominal aortas in streptozotocin-induced diabetic apoE-deficient mice (84). Alagebrium treatment significantly attenuated plaque area or complexity within the thoracic and abdominal aortas and inhibited accumulation of AGE-modified collagens in the aortas with reduced expression of RAGE and profibrotic cytokines, TGF-β and connective tissue growth factor in streptozotocin-induced diabetic apoE-deficient mice as well (84). In addition, alagebrium reduced transdifferentiation of proximal tubules in the diabetic kidneys in association with reduced tubular AGE and TGF-β levels. Alagebrium treatment also decreased AGE and collagen accumulation in the diabetic kidneys, inhibited glomerulosclerosis and tubulointerstitial injury and retarded albumin excretion rate in streptozotocin-induced diabetic rats. These rats were associated with reduced renal expression of RAGE,

TGF-β and connective tissue growth factor (85).

Pigment Epithelium-Derived Factor

Pigment epithelium-derived factor (PEDF), a glycoprotein that belongs to the superfamily of serine protease inhibitors with potent neuronal differentiating activity, inhibits vascular hyperpermeability, inflammatory, thrombotic and fibrotic reactions in diabetic nephropathy and retinopathy partly by suppressing the AGE–RAGE axis, which could potentially be exploited as a therapeutic option for the treatment of vascular complications in diabetes (86–89).

Renin-Angiotensin System Inhibitors

The interaction of the renin-angiotensin system (RAS) and AGE–RAGE axis has been proposed (90). We have found that angiotensin II (Ang II) potentiates the deleterious effects of AGEs on pericytes by inducing RAGE protein expression (91). *In vivo*, AGE injection stimulated RAGE expression in the eye of spontaneously hypertensive rats, which was blocked by telmisartan, an Ang II type 1 (AT1) receptor blocker. *In vitro*, Ang II-AT1 receptor–mediated oxidative stress generation elicited RAGE gene upregulation in retinal pericytes through NF-κB activation. Furthermore, Ang II augmented AGE-induced pericyte apoptosis, the earliest hallmark of diabetic retinopathy (2). In addition, the AGE–RAGE axis stimulates Ang II production or AT1 receptor overexpression in renal constituents, further deteriorating diabetic nephropathy (92–94).

Dipeptidyl Peptidase-4 Inhibitors and Incretins

There is a pathological crosstalk between the AGE–RAGE system and dipeptidyl peptidase-4 (DPP-4)-incretin axis in the pathogenesis of vascular complications in diabetes (95,96). Glucagon-like peptide-1 directly acts on endothelial cells, mesangila cells and proximal tubular cells via the glucagon-like peptide-1 receptor, and glucagon-like peptide-1 could work as an antiinflammatory and antioxidative agent against AGEs by reducing RAGE expression via activation of cyclic AMP pathways (95,96). Moreover, we have recently found that AGE–RAGE–induced oxidative stress generation stimulates the release of DPP-4 from endothelial cells, which could act on endothelial cells in an autocrine manner via the interaction with mannose 6-phosphate insulin-like growth factor II receptor, further potentiating the deleterious effects of AGEs (97). DPP-4 deficiency or an inhibitor of DPP-4 protects against experimental diabetic nephropathy in a glucose-lowering–independent manner, where benefficial effects were partly mediated by suppression of the AGE–RAGE axis (98,99).

AGE-Aptamer

Aptamers are short, single-stranded DNA or RNA molecules that can bind with high affinity and specificity to a wide range of target proteins (100,101). We have recently found that a high-affinity DNA aptamer directed against AGEs (AGE-aptamer) inhibits glomerular hypertrophy and extracellular matrix protein accumulation, decreases urinary excretion levels of albumin and prevents renal dysfunction in type 2 diabetic animals (100). In this study, AGE-aptamer directly bound to AGEs and resultantly blocked the binding of AGEs to RAGE, and continuous infusion of AGE-aptamer dramatically decreased AGE levels in the glomeruli of diabetic mice (100). Therefore, it is conceivable that AGE-aptamer might decrease the glomerular accumulation of AGEs via the blockade of RAGE-induced, oxidative stress–mediated AGE formation in the kidney. In addition, turnover rate of aptamer-bound AGEs by THP-1 macrophages was increased. Therefore, AGE-aptamer could enhance the elimination of AGEs from the body through increased turnover by macrophages.

MEASURING SERUM LEVELS OF AGES AND ITS CLINICAL UTILITY

The facts that AGEs could reflect cumulative diabetic exposure and play a role in the pathogenesis of diabetic vascular complications led us to speculate that circulating levels of AGEs could be a biomarker of vascular injury and organ damage in patients with diabetes. To address the issue, we began to develop an enzyme-linked immunosorbent assay (ELISA) system for measuring serum levels of toxic AGEs in humans.

In the article in *Molecular Medicine* in 2001, we produced five specific antibodies for noncarboxymethyllysine AGEs that recognized the immunoreactive types of AGEs (glucose-, glyceraldehyde-, glycolaldehyde-, methylglyoxal- and glyoxal-derived AGEs) (10). We found that (a) five distinct classes of AGE structures circulate in the blood of individuals with diabetic nephropathy on hemodialysis (DM-HD), (b) neurotoxic effects of serum fraction from DM-HD containing various AGEs structures are completely neutralized by the addition of antibodies raised against glyceraldehyde-derived AGEs, and (c) this type of AGE mimics the deleterious effects of AGE-rich serum purified from DM-HD on endothelial cells (85,102). Furthermore, because of the stronger binding affinity to RAGE (103), glyceraldehyde-derived AGEs are considered to be more toxic than glucose-derived AGEs. Hence, we focused on glyceraldehyde-derived AGEs, developed a specific ELISA for this type of AGE and examined the clinical utility of measuring glyceraldehyde-derived AGEs for evaluating disease activity in patients with various diabetes- and aging-associated disorders.

So far, we have found that glyceraldehyde-derived AGE levels are (a) correlated with a soluble form of RAGE that could reflect tissue RAGE expression in both nondiabetic and diabetic subjects (104–107), thus suggesting a marker of the activation of AGE–RAGE axis; (b) associated with low-density lipoprotein cholesterol levels and thrombogenic markers such as plasminogen activator inhibitor-1 and fibrinogen in a general population (108–110); (c) elevated under oxidative stress, chronic kidney disease and/or diabetic conditions and corre-

lated with inflammatory biomarkers such as monocyte chemoattractant protein-1, the soluble form of vascular cell adhesion molecule-1 and ADMA (106,107,111–113); (d) increased in nonalcoholic steatohepatitis (NASH) patients and associated with insulin resistance in both NASH subjects and an non-NASH general population (114–116); (e) correlated with serum PEDF and DPP-4 levels, markers of insulin resistance (117,118); (f) associated with visceral and subcutaneous adipose tissue inflammation and decreased adiponectin levels (114,119); (g) correlated with vascular inflammation and endothelial dysfunction in high-risk patients (120,121); (h) inversely associated with number and migratory activity of EPCs (122), thus suggesting the involvement of this type of AGEs in impaired endothelial cell repair; and (i) significantly associated with plaque progression in patients with acute coronary syndrome (123). Moreover, atrovastatin, pioglitazone and α-glucosidase inhibitor have been shown to significantly decrease serum levels of glyceraldehyde-derived AGEs, which are associated with reduced biomarker levels for organ damage in diabetic, chronic kidney disease or NASH subjects (120,124–128).

CONCLUSIONS

Glyceraldehyde, which could be derived from glucose metabolism, is not a major sugar *in vivo* and its incubation with proteins will generate a large number of AGEs; in addition, there is some criticism that measurement of AGEs using liquid chromatography–tandem mass spectrometry technique may produce different results to that using ELISA (129). However, our series of studies have suggested that serum levels of glyceraldehyde-derived AGEs might be a novel biomarker for insulin resistance and vascular injury and may predict future cardiovascular events in diabetes. Measuring this type of AGEs by specific ELISA could identify high-risk patients and may provide us variable information for treatment decision-making in the future.

In addition, we have recently found that AGE-aptamer raised against glyceraldehyde-derived AGEs, but not control-aptamer, binds to glyceraldehyde-derived pyridinium (GLAP) and the 3-hydroxy-5-hydroxymethyl-pyridinium adduct and subsequently blocks the interaction of GLAP with RAGE. Moreover, a GLAP-evoked increase in oxidative stress generation and upregulation in RAGE as well as inflammatory reactions were significantly inhibited by treatment with AGE-aptamer but not control-aptamer (130). These findings suggest that GLAP is a target compound for AGE-aptamer and might be a main glyceraldehyde-related AGE structure that interacted with RAGE and elicited oxidative, inflammatory and thrombogenic reactions in endothelial cells. GLAP was only formed in glyceraldehyde-modified bovine serum albumin, not in another sugar-modified one, thus supporting our speculation (131). So, development of a simple, specific, reliable assay system for GLAP would be desired, and it should be an important issue in the feature to examine whether serum levels of GLAP may be a novel biomarker for vascular damage and CVD in diabetes.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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