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EDITORIAL

Role of advanced glycation end products in cardiovascular disease

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Abstract

Advanced glycation end products (AGEs) are produced through the non enzymatic glycation and oxidation of proteins, lipids and nucleic acids. Enhanced formation of AGEs occurs particularly in conditions associated with hyperglycaemia such as diabetes mellitus (DM). AGEs are believed to have a key role in the development and progression of cardiovascular disease in patients with DM through the modification of the structure, function and mechanical properties of tissues through crosslinking intracellular as well as extracellular matrix proteins and through modulating cellular processes through binding to cell surface receptors [receptor for AGEs (RAGE)]. A number of studies have shown a correlation between serum AGE levels and the development and severity of heart failure (HF). Moreover, some studies have suggested that therapies targeted against AGEs may have therapeutic potential in patients with HF. The purpose of this review is to discuss the role of AGEs in cardiovascular disease and in particular in heart failure, focussing on both cellular mechanisms of action as well as highlighting how targeting AGEs may represent a novel therapeutic strategy in the treatment of HF.

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INTRODUCTION

Advanced glycation end products (AGEs) are a heterogenous group of molecules that are generated through non-enzymatic glycation and oxidation of proteins, lipids and nucleic acids[1]. They alter tissue function and mechanical properties through crosslinking intracellular as well as extracellular matrix proteins[2-6] and through binding to their cell surface receptor, receptor for AGEs (RAGE), they are capable of modulating multiple cellular processes[7-9]. Enhanced formation and accumulation of AGEs has been reported to occur in conditions such as diabetes mellitus (DM) as well as in natural aging, renal failure and chronic inflammation[10-12].

*In vivo d*etectable AGEs include three main groups: (1) Fluorescent cross-linking AGEs such as pentosidine

and crossline[13]; (2) Non-fluorescent cross-linking AGEs such as imidazolium dilysine cross-links named either glyoxal lysine dimer or methylglyoxal lysine dimer result from reactions taking place between glyoxal derivatives and lysine residues[14]; (3) Non-cross-linking AGEs such as N-carboxymethyllysine (CML)[15].

In patients with diabetes, cardiovascular complications are the principal cause of morbidity and mortality and account for up to 65% of diabetic fatalities[16]. It has been reported that 33% of diabetic patients on insulin therapy will have died from cardiovascular disease by the age of 50 years[17]. It is thought that AGEs have a central role in the pathophysiological processes that lead to the development of such cardiovascular complications observed in diabetes. This review highlights how AGEs are formed, summarises the evidence for the role of AGEs in the development of cardiovascular complications in diabetic patients, as well as their underlying mechanisms of action and finally we review the potential of anti-AGE therapies for their use in clinical practice.

MAILLARD REACTION AND AGE SYNTHESIS

AGE are a spectrum of heterogeneous compounds that are derived from proteins, lipids and nucleic acids that are glycated or oxidized non-enzymatically in a process termed the "Maillard reaction"[18]. D-glucose plays a primary role in glycation of proteins *in vivo* due to its high concentration in human plasma[19]. The Maillard reaction starts as a reaction between the carbonyl group of a reducing sugar such as glucose and the amino acid of proteins, lipids or nucleic acids leading to the production of an unstable compound known as a "Schiff base". This step is reversible and usually takes a few hours to occur. Over weeks the Schiff base turns into a more stable compound called the "Amadori" product through various molecular rearrangements. Over months and years, the Amadori products undergo further structural changes through a series of reactions such as oxidation, dehydration and degradation to finally yield highly stable AGE compounds[20,21] (Figure 1).

Highly reactive dicarbonyl compounds are generated during the conversion of Amadori products to AGEs. Methylglyoxal and 3-deoxyglucosone are the best known AGE dicarbonyl precursors[22-24]. *In vivo*, CML is the most abundant form of AGEs and is characterized by its highly antigenic nature[25,26].

It has been reported that non-enzymatic glycation of proteins and formation of AGEs affects the protein physiological functions and is capable of inducing enzyme inactivation[27]. The influence of such modifications on protein structure has been far less investigated. Recently, using nuclear magnetic resonance (NMR), Howard *et al*[28] have showed that glycation of a model helical peptide from human serum albumin (HSA) by glucose caused distortion of the helical structure of the protein at the point of glycation. Another protein structural study for the changes induced by non-enzymatic glycation in the secondary and tertiary protein structure of HSA showed that the in general protein structure was unaltered. Instead the change was only limited to local regions after glycation. This was revealed using fluorescence-dependant methods[29].

AGES AND CARDIOVASCULAR DISEASE

AGEs are produced normally in the body and they accumulate by age and are considered not only as biomarkers of senescence but they correlate inversely with left ventricular ejection fraction (EF) and can predict the outcome of cardiac surgery in elderly patients. A direct linear correlation between CML levels in the pericardial fluid and the age of the patients was shown where mean CML level of 260.8 ± 19.7 ng/mL was detected in patients with mean age 52.2 ± 1.3 years, mean CML level of 293.4 ± 18.8 ng/mL was identified in patients with mean age 65.8 ± 0.6 years and mean CML level of 357.0 ± 28.3 ng/mL was recorded at mean age 75.1 ± 0.6 years[30].

In addition, AGEs accumulate at a much higher rate in diabetics than in normal population. AGEs serum levels are much higher in diabetic patients when compared to normal non diabetic population. AGE serum levels have been reported in a number of clinical studies involving diabetic patients compared to healthy individuals. AGE levels were recorded as 7.4 U/mL in diabetic patients *vs* 4.2 U/mL in normal population, whereas CML levels reached 15.6 U/mL in diabetics *vs* 8.6 U/mL in nondiabetics. Serum AGEs were found to be significantly elevated in diabetic patients with coronary heart disease (CHD) (8.1 U/mL) *vs* diabetics without CHD (7.1 U/mL)[31]. In another study concerned with AGE tissue levels and the severity of diabetic nephropathy, the average AGE content in arterial wall collagen of diabetics was significantly higher than that of samples obtained from nondiabetic patients (14.5 ± 5.2 U/mg *vs* 3.6 ± 1.5 AGE U/mg). Furthermore, it was revealed that AGE content in renal tissues obtained from diabetic patients with end-stage renal disease is as twice as AGE content in tissue of diabetic patients without renal disease (21.3 ± 2.8 U/mg *vs* 11.5 ± 1.9 AGE U/mg)[32]. Serum levels of pentosidine were found to be significantly higher in patients with diabetes than in nondiabetic normal controls (64.4 ± 21.0 μg/L *vs* 22.8 ± 7.0 μg/L). Moreover, serum pentosidine levels were significantly higher in diabetic patients with cardiovascular disease than in those without (72.3 ± 23.7 μg/L *vs* 62.3 ± 19.8 μg/L) and they were found to correlate with increased arterial wall stiffness in diabetic patients[33].

AGES AND CARDIOVASCULAR COMPLICATIONS OF DIABETES

As mentioned above, several studies have shown a positive association between serum and tissue AGEs and macro and microvascular complications of diabetes[31,34-39].
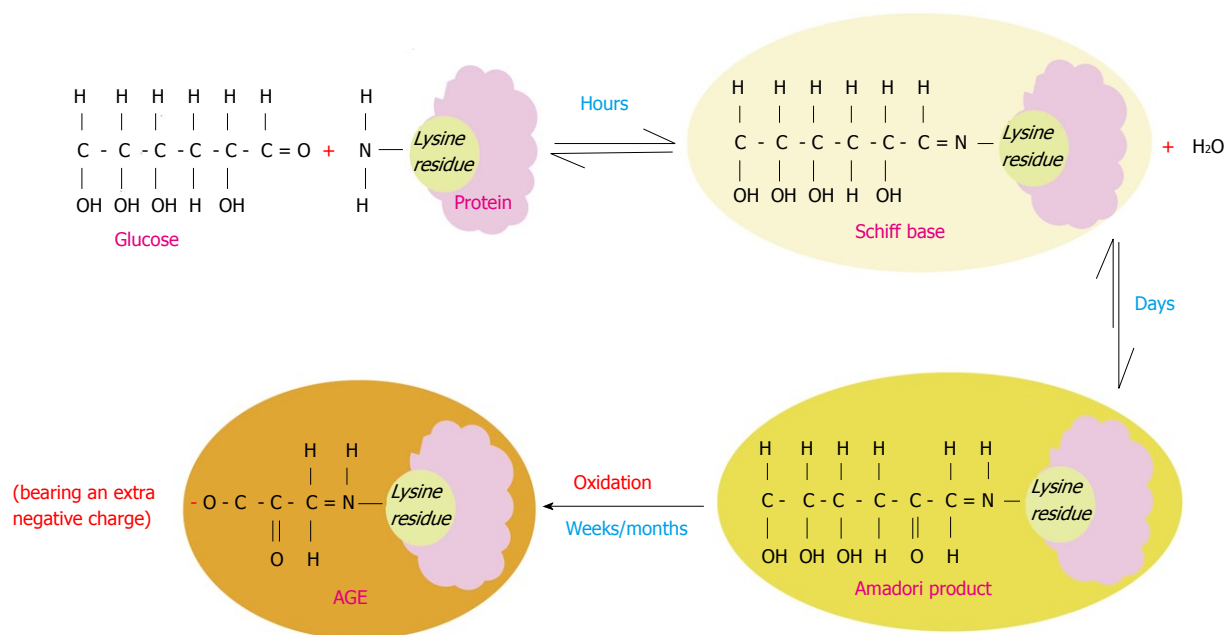
Figure 1 Maillard reaction. Schematic illustration of the Maillard process that starts by a non-enzymatic reaction of a protein lysine residue (as an example of the most common residues that get glycated) and glucose with consequent loss of a water molecule. This is a reversible reaction that takes place over a few hours leading to the formation of an unstable compound (Schiff base). The latter undergoes molecular rearrangements over a period of days yielding a more stable compound known as (the Amadori product). Over months/years, the Amadori product turns to a very stable compound known as advanced glycation end product (AGE) through a series of reactions such as oxidation. Therefore AGE molecules acquire more negative charges. AGEs are irreversible once they are formed. The Maillard reaction leads to denaturation and browning of the modified proteins.

The following sections overview how serum AGE levels correlate with the development and the progression of several cardiovascular complications of diabetes.

AGEs, coronary heart disease and diabetic heart failure

Elevated serum levels of AGEs such as CML have been documented in type 2 diabetic patients with coronary heart disease[31]. AGEs have been detected in atheromatous lesions in coronary arteries of diabetic patients, suggesting a role of AGEs in the accelerated development of atherosclerosis reported in diabetics[40]. Moreover, serum AGE levels have been shown to be a biomarker for the severity of coronary artery atherosclerosis in type 2 diabetic patients[27] independent from other well known risk factors such as hypertension, hyperlipidaemia and smoking. Higher serum AGE levels were detected in type 2 diabetic patients with obstructive coronary artery disease in comparison to diabetics with non-obstructive coronary disease and AGE serum levels were found to correlate with the degree of the coronary atherosclerosis in those with obstructive coronary disease[41]. AGE levels have also been reported to influence the success rate of coronary artery revascularization in patients with diabetes. Elevated serum AGE levels in patients undergoing percutaneous coronary intervention has been shown to be an independent risk factor for restenosis in diabetic patients[42]. Following cardiac surgery, serum AGE levels also inversely correlate with left ventricular ejection fraction and are associated with prolonged ventilation time and prolonged stays on the Intensive Care Unit[43,44].

Other studies have demonstrated that elevated serum AGE levels have been shown to predict mortality due to coronary heart disease in female patients with type 2 diabetes followed up for 18 years[31]. Similarly, increased serum AGE levels in type 1 diabetes have been shown to correlate with incident fatal as well as nonfatal cardiovascular events independent from other known cardiovascular risk factors such as age, body mass index, smoking, hypertension and hyperlipidaemia[45].

The risk of development of cardiovascular disease is increased by a factor of 2-4 fold in diabetic patients[46]. DM is considered to be an independent risk factor for incident heart failure (HF)[47-49]. In the Framingham study (between the ages 45-74), the risk of developing HF was higher in diabetic males (2.4:1) and diabetic females (5:1) independent of age, obesity, hypertension, hyperlipidaemia and coronary artery disease[50].

Elevated serum levels of AGEs in patients with diabetes accelerate the development and progression of heart failure both indirectly through their vascular effects (coronary dysfunction, atherosclerosis and thrombosis) and directly through direct actions on the myocardium. In the former, AGEs binding to their cell surface receptor (RAGE) on endothelial cells, smooth muscle cells and monocytes thereby inducing a wide range of signaling pathways that trigger inflammation, atherogenesis and vasoconstriction leading to coronary dysfunction, atherosclerosis and thrombosis[14]. On the other hand, AGEs have been reported to have a direct effect on the myocardium independent of effects on the vascular tree, mediated in part *via* cross linking of extracellular cardiac proteins and through actions mediated by AGE

receptors expressed on the myocardium[5,6,51]. AGEs have been implicated in the development of both systolic and diastolic cardiac dysfunction in diabetics which may explain the increased prevalence of heart failure in diabetic patients[14,52]. It was shown by Steine *et al*[53] that serum AGE levels and duration of diabetes can predict systolic strain (as evaluated by doppler tissue imaging) in patients of type 1 diabetes. Moreover, Kilhovd *et al*[31] showed that parameters of left ventricular diastolic dysfunction, e.g., delayed isovolumetric relaxation time and reduced LV end-diastolic diameter correlate with elevated plasma AGE levels in type 2 diabetic patients. Similarly, Willemsen *et al*[54] showed that increased tissue levels of AGEs (as detected by increased skin autofluorescence) in diabetic heart failure patients are independently associated with diastolic dysfunction and consequently reduced exercise capacity in those patients in comparison to non-diabetic heart failure patients.

Serum AGE levels have been demonstrated as independent predictors of both the severity and prognosis in heart failure patients[14,55]. Plasma pentosidine levels, one of the crosslinking AGEs, has been shown to be an independent predictor of both re-hospitalization and mortality in heart failure patients independent from other known risk factors such as brain natriuretic peptide (BNP), age, renal function and New York Heart Association (NYHA) functional class[55]. Similarly, plasma levels of N-CML, one of the most prevalent antigenic AGEs in sera of diabetic patients, have been shown to correlate with NYHA functional class and predict outcomes in patients with systolic heart failure[14].

Another study conducted by Neeper *et al*[56] showed that serum levels of soluble RAGE (sRAGE) are markers of the development and the progression of heart failure in diabetic and non-diabetic patients. The term sRAGE includes both cleaved RAGE (cRAGE) and endogenously secreted RAGE (esRAGE). The former is cleaved RAGE from cell surface by action of metalloproteinases that are increased in HF. This form lacks the V-domain and is therefore unable to neutralize AGEs. The latter is an endogenously secreted RAGE that helps to neutralize serum AGEs. Both higher serum levels of cRAGE and lower serum levels of esRAGE correlate with the severity of cardiac dysfunction, severity of symptoms and clinical outcomes in patients with heart failure[56]. Similar findings have been recorded in a number of other HF studies. For instance, Koyama *et al*[57] have shown that serum levels of sRAGE correlate with NYHA functional class and that they are particularly higher in HF patients with preserved EF suggesting that they have a role in diastolic dysfunction. Furthermore, Koyama *et al*[57] reported that sRAGE levels are independent prognostic factors in HF[57]. Similarly, Raposeiras-Roubín *et al*[58] also demonstrated that sRAGE is a highly sensitive and specific marker of prognosis of HF patients.

Other studies have yielded conflicting observations about the role of AGEs in the development of heart failure whether directly or indirectly. For instance, in another study, the authors demonstrated that increased serum sRAGE levels are associated with increased severity of HF particularly in patients with an ischemic cause of heart failure although this correlation was shown to be independent of AGE serum levels[59]. Similarly, in the study of Campbell *et al*[60], left ventricular biopsies were taken from pre-diabetic, type 2 diabetic and control subjects undergoing coronary heart bypass surgery to assess myocardial fibrosis and myocardial expression of the AGE N-CML and the RAGE. All the patients had similar degrees of coronary disease with no previous history of myocardial infarction or heart failure although diabetic patients exhibited diastolic dysfunction on echocardiography. The study demonstrated no significant differences in either myocardial AGE or RAGE expression between patient groups or the control group suggesting that myocardial AGE/RAGE expression was not an important factor in diastolic dysfunction development in diabetes. In a similar study in which endomyocardial biopsies of failing hearts of diabetic patients were studied, no increase in myocardial fibrosis or myocardial CML expression was observed in cases with preserved left ventricular ejection fraction although both myocardial fibrosis and CML expression were elevated in cases with significant systolic dysfunction[60].

## AGEs and other forms of macrovascular diseases

Serum levels of AGEs were also shown to correlate with the presence of other forms of macrovascular pathology as carotid stenosis and peripheral artery occlusive disease. Serum levels of the esRAGE that can neutralize circulating AGEs were found to be inversely proportionate to carotid artery intima-media thickness in type 1 diabetic patients[61]. The same was reported in type 2 diabetic patients as well as non-diabetic cases[62]. Higher expression of the RAGE was detected in carotid artery plaques and was shown to be associated with enhanced inflammatory reactions[63].

Type 2 diabetic patients with peripheral artery disease exhibited higher levels of serum AGEs *vs* the non-diabetic subjects[39]. A tight link has been shown between AGE plasma levels and pulse pressure in type 1 diabetic patients as was reported in the EURODIAB Prospective Complications Study[64].

AGEs and microangiopathy

Serum and tissue levels of AGEs are predictors of the development of microvascular complications in diabetes. For example in type 1 diabetes control and complications trial, increased AGE levels in skin biopsies predict the development of diabetic microvascular complications, e.g., retinopathy and nephropathy[13,65]. AGE/RAGE interaction has been suggested as a possible mechanism coupling microangiopathy with diabetic nephropathy, retinopathy and neuropathy[43].

Diabetic nephropathy: Correlation between the levels of skin collagen AGEs particularly CML and the devel-
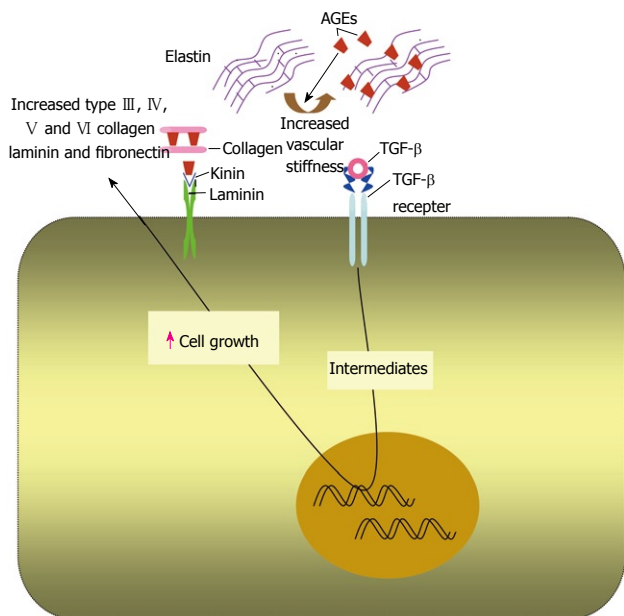
Figure 2 Effects of advanced glycation end products on extracellular matrix proteins. In extracellular matrix, advanced glycation end products (AGEs) form on different molecules as collagen, laminin and elastin. This alters the physiological properties of the matrix and increases its stiffness. AGEs upregulate transforming growth factor (TGF)-β that increases the production of extracellular matrix components by binding to its receptor.

opment and worsening of microvascular complications including nephropathy in type 1 diabetic patients was initially reported by Genuth *et al*[65]. Similarly, in type 2 diabetes skin AGEs (skin autofluorescence) were shown to be potent independent predictors of evolution of microvascular complications including nephropathy[66]. In diabetes, the kidney is an important site for AGE accumulation but also contributes to the increased levels of AGEs in the serum of diabetic patients since the kidney is the main site for AGE clearance[67]. Diabetic animal models exhibit higher levels of AGE deposition in their kidneys[68] which has been linked to renal structural alterations reported in diabetic nephropathy such as glomerular basement membrane thickening, glomerulosclerosis, mesangial expansion and tubulointerstitial fibrosis. Similar histological findings have been also documented in murine models injected with AGE-albumin[69].

Diabetic retinopathy: Accumulation of AGEs especially CML in retinal blood vessels of type 2 diabetic patients has been reported. It has been also documented that the levels of AGEs correlated with the severity of the retinopathy[70,71]. Infusion of AGE-albumin in non-diabetic animals has shown that AGEs localize both inside and around the pericytes co-localizing with the AGE receptor causing thickening of the basement membrane and destruction of the blood retinal barrier[72,73]. *In vitro* exposure of retinal cells to AGEs induced upregulation of vascular endothelial growth factor (VEGF) that may contribute to retinal neovascularisation reported in diabetic retinopathy[74].

Diabetic neuropathy: Both peripheral and autonomic neuropathies have been shown to correlate with AGE levels even before neuropathy becomes clinically evident[75]. Skin autofluorescence reflecting AGE bound collagen levels has been reported to have a tight link with the degree of diabetic neuropathic foot ulceration[76]. AGEs have been shown to accumulate in peripheral nerves of diabetic patients and they were shown to colocalize with RAGE receptor in endoneurial, perineurial and epineurial blood vessels[77].

MECHANISMS OF ACTIONS OF AGES

AGEs mediate their tissue effects through three main mechanisms: (1) Cross linking extracellular (matrix) proteins thereby affecting tissue mechanical properties[51]; (2) Cross linking intracellular proteins thus altering their physiological functions[5,6]; and (3) Binding to their cell surface receptor RAGE to inducing multiple intracellular signalling cascades[56]. In the following sections these mechanisms will be discussed.

Crosslinking tissue proteins

Cross linking extracellular matrix proteins: AGE formation is a process of chronicity usually affecting long-lived proteins. Extracellular matrix proteins especially collagen type Ⅳ that is involved in basement membrane structure, are more prone to advanced glycation due to their long turnover[20,78]. Advanced glycation and crosslinking of other extracellular matrix proteins, e.g., collagen I and elastin render them stiffer and less susceptible to proteolytic digestion[51] (Figure 2). This may contribute to the observed increase in vascular stiffness reported in diabetes and old age[20,51,79]. In addition, cross linking myocardial collagen with AGEs has been suggested to cause myocardial stiffness and diastolic dysfunction in diabetic patients[51,80].

AGEs alter the structure of low density lipoproteins (LDL) through glycation therefore preventing their clearance from the circulation *via* the normal elimination route, i.e., uptake by the endothelial cells. Instead they are uptaken by the blood monocytes leading to foam cells that contribute to the pathogenesis of atherosclerosis[81,82].

Cross linking intracellular proteins: AGEs have been shown to be implicated in crosslinking of intracellular proteins and hence altering their physiological properties and functions. For instance AGEs were shown to cross link the domains of both the Ryanodine receptor[5] and SER-CA2a[6] in cardiomyocytes leading to alterations in calcium homeostasis reported in diabetic cardiomyopathy[83,84].

RAGE dependant effects of AGEs

RAGE receptor: RAGE belongs to the immunoglobulin superfamily of receptors[56]. It has been reported that RAGE gene is located on chromosome 6 in humans between genes coding for class Ⅱ and class Ⅲ major histocompatibility complexes[85]. The RAGE promoter
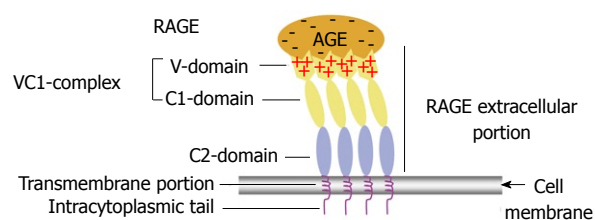
Figure 3 Receptor for advanced glycation end product (i.e., colon) receptor structure and its functional implication in binding different advanced glycation end products. A diagrammatic illustration of the structure of the receptor for advanced glycation end product (RAGE) showing that it is composed of an extracelluar portion, a transmembrane portion and an intracytoplasmic tail. The extracelluar portion comprises three domain V, C1 and C2. The first two are believed to work together as a single functional complex (VC1) whereas the C2 domain remains attached to the VC1 complex but works independently from it. The diagram also illustrates how multiple RAGE receptors polymerise within the cell membrane to facilitate high affinity binding of the positively charged V domain with the negatively charged advanced glycation end products (AGEs) independent of their chemical structure. That is why RAGE is considered one of the pattern recognition receptors.

has been shown to possess nuclear factor kappa B (NF-κB) binding sites, hence linking RAGE expression to the inflammatory cascade[86].

RAGE is a multiligand receptor with advanced glycation end products being identified as its first known ligands. Hereafter, multiple other RAGE ligands have been revealed including; high mobility group protein box-1[21], some members of the S100 protein family[87] amyloid β[88,89] and fibrillar protein aggregates[90,91]. Therefore, RAGE has an important role in the pathogenesis of the diseases induced by such ligands, e.g., inflammation, tumours, neurodegeneration and amyloidoses[21,88,92,93].

RAGE structure: Full length RAGE comprises three domains, an extracellular domain of 332-amino acids arranged as a single "V"-type immunoglobulin-like (variable) domain with subsequent two "C"-type (constant) domains[89,94]. Modern biochemical techniques have revealed evidence suggesting that both the V and C1 domains of RAGE function together as an incorporated single structural unit for the binding of some ligands. In contrast, C2 RAGE domain is suggested to function completely independent from the VC1 complex while remaining attached to it through a flexible hinge (Figure 3). It has been deduced from experimental work that different RAGE ligands interact with one or more of its domains[19].

RAGE has a single transmembrane domain and a highly charged cytosolic tail formed of 43 amino acids (Figure 3). The cytosolic tail is vital for RAGE ligands to activate intracellular signalling cascades. RAGE isoforms in which the cytosolic tail was absent, bind AGEs but fail to elicit intracellular signaling on ligand binding[95]. It has recently been shown that for some RAGE actions, its tail binds directly to a cytoplasmic molecule known as the mammalian diaphanous-1 which is essential for eliciting phosphorylation/activation steps needed for initiation of the signalling cascade[96].

Despite the wide variation in their chemical structure,

AGEs bind to the V domain of the RAGE receptor[94]. The fact that RAGE recognizes a class of biochemically heterogeneous ligands such as AGEs categorizes RAGE as one of the pattern recognition receptors[97-99] that identifies common features or patterns rather than a specific ligand. Though AGEs exhibit diverse chemical structure, however, they have some common general characteristics. First, all AGE modified proteins demonstrate a net negative charge that accumulates during their formation by glycation and oxidation[100,101]. The second main feature is that modifications of proteins by AGEs lead to creation of multiple covalent cross-links resulting in higher molecular mass molecules (multimers). This ligand geometry is thought to be important for RAGE activation[100].

Recent molecular structure revealing technologies including X-ray crystallographic, NMR together with conventional biochemical data have illustrated that the unusual ability of the RAGE receptor to bind different AGEs lies in its extracellular portion (VC1 ectodomain) where it has been suggested that the ligand binding is triggered mainly by electrostatic interactions between the positively charged surface of this subunit and negatively charged ligands (Figure 3)[102]. The V domain has been shown by NMR to exhibit three distinct areas for mediating AGE-V domain interactions. Such areas are situated in the positively charged regions of the V domain. The first interaction surface includes strand C and loop CC', the second interaction surface comprises strand C', strand F and loop FG, and the third interaction one consists of strand A' and loop EF[101].

The second main point to be considered here is that studies based on using a fluorescence-labelled receptor demonstrated that RAGE does not float as one molecule in the plasma membrane but instead multiple RAGE receptors aggregate to form receptor assemblies[19,101]. Therefore, RAGE exists in constitutive multimers that usually includes four molecules or more within the plasma membrane[101,103]. RAGE multimers are thought to display a parallel orientation with VC1 subunits exhibiting side to side contacts (Figure 3) as revealed in protein crystals. Therefore it has been speculated that the general multimeric structure adopted by AGE modified proteins together with numerous AGE-modified side chains are essential requirements for preserving the receptor assemblies' stability needed for activation of the receptor[100] probably in a similar way to that previously described for the receptor tyrosine kinases where the intracellular domains of the receptor that possess intrinsic kinase activity must come very close together in a particular orientation that facilitates cross-phosphorylation of the domains and hence commencement of the signalling cascade[104].

RAGE isoforms: Despite the presence of a single gene coding for RAGE, there are several splice variants of this gene with three main RAGE isoforms having been identified: full length RAGE, dominant-negative RAGE (*DN-*
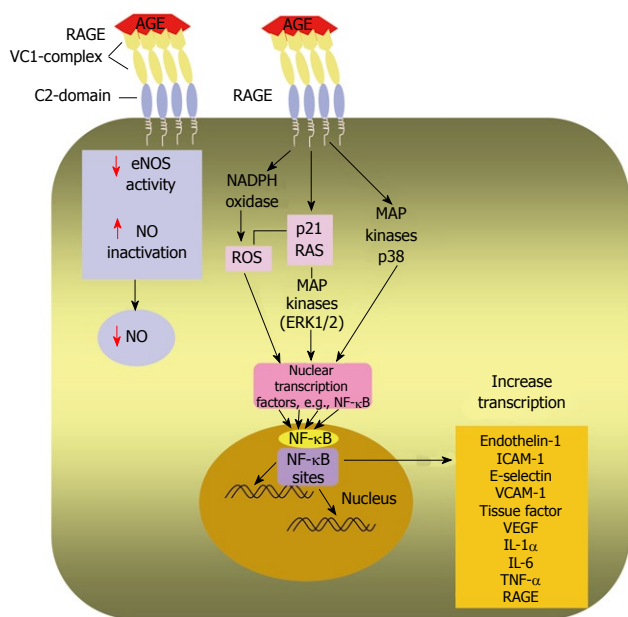
Figure 4 Intracellular effects of AGEs after AGE/RAGE binding. Diagrammatic representation of AGE/RAGE interaction on the surface of an endothelial cell leads to transduction of a signalling cascade; activates nicotinamide adenine dinucleotide phosphate oxidase and enhances ROS production and phosphorylate p21 RAS and MAPKs. Moreover, the AGE/RAGE interaction induces signalling through activation of p38 MAPK. A main step in AGE/RAGE signalling is activation of NF-κB and its translocation to the nucleus, where it enhances transcription of target genes as endothelin-1, ICAM-1, E-selectin and tissue factor. Hence, AGE/RAGE binding triggers an inflammatory cascade. AGE may decrease NO availability by reducing eNOS activity and by inactivating NO. AGE: Advanced glycation end-product; RAGE: Receptor for advanced glycation end products; ROS: Reactive oxygen species; MAPKs: Mitogen-activated protein kinases; ERK1/2: Extracellular signal-regulated kinase 1/2; NF-κB: Nuclear factor kappa B; ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; VEGF: Vascular endothelial growth factor; IL-1α: Interleukin-1α; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide.

RAGE) and endogenous soluble RAGE (e*s-RAGE*)[105]. Full length RAGE is composed of the whole three domains; extracellular, trans-membrane and intracellular domains. DN-RAGE has extracellular and transmembrane domains, but no cytosolic tail. Endogenous soluble RAGE possesses the extracellular domain only so it is found free in the circulation. The only RAGE isoform that is capable of eliciting intracellular signalling upon interacting with its ligands is the full length RAGE as this is induced through its cytosolic tail. However, the other two isoforms; DN-RAGE and esRAGE help in clearance and neutralisation of circulating AGEs by competing with full length RAGE in binding them[56,105].

RAGE tissue distribution: RAGE expression has been detected in a number of cells including endothelial cells, smooth muscle cells, monocytes/macrophages, T lymphocytes, cardiomyocytes, glomerular podocytes, dendritic cells, neurons of the central and peripheral nervous systems and transformed cells[106]. Generally, there is a low expression of RAGE in tissues. However, it becomes up-regulated in an environment rich with its

ligands as in the case of diabetes or aging. RAGE expression was higher in endothelial cells, monocytes and smooth muscles in diabetic vascular tissue[107].

INTRACELLULAR EFFECTS OF AGE-RAGE BINDING ON CARDIOVASCULAR SYSTEM

RAGE activation by high serum and tissue levels of AGEs induces multiple intracellular signalling pathways (Figure 4) that have been implicated in pathogenesis of serious diabetic complications such as enhanced atherosclerosis, cardiovascular disease, nephropathy and chronic inflammation[9,10,108-110]. It has been documented that circulating AGEs bind with endothelial RAGE resulting in endothelial dysfunction *via* activation of a number of signaling pathways, e.g., activation of nicotinamide adenine dinucleotide phosphate oxidase that enhances the production of reactive oxygen species (ROS)[108]. ROS have been shown to play a pivotal role in causing major cardiovascular damage in diabetes through modifying the structure of cellular proteins, lipids and nucleic acids and therefore altering their physiological roles[111].

In addition, it has been reported as well that AGE-RAGE engagement increases phosphorylation of p21ras, the mitogen-activated protein kinases, extracellular signal-regulated kinase 1/2 and p38, and activates GTPases Cdc42 and Rac, ultimately inducing activation and translocation of NF-κB from cytoplasm to the nucleus where it starts transcribing its target set of genes[112]. The latter include, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, VEGF, endothelin-1, tissue factor, E-selectin, thrombomodulin and proinflammatory cytokines, including interleukin (IL)-1α, IL-6 and tumor necrosis factor-α[113,114]. The above mentioned cytokines and adhesion molecules have central roles in both inflammation and atherosclerosis[108,115].

ANTI-AGE THERAPIES

Therapies that target AGEs can be classified into two main categories, the ones that prevent the formation of AGEs and others that breakdown the already formed AGEs.

Prevention of AGEs formation

Aminoguanidine (AG), is a hydrazine compound that inhibits AGE formation *via* trapping of carbonyl intermediates (early active glycation products) and hence preventing modification of nucleophilic residues in proteins[116,117]. In a placebo-controlled, randomized trial involving type 1 diabetes patients, the use of aminoguanidine hindered reduction in glomerular filtration rate, reduced 24-h urinary proteinuria and prevented the deterioration of retinopathy. However, it did not influence time-to-doubling of serum creatinine[118]. Being an NOS inhibitor, which is an essential renovasodilator, this may offset some of AG benefits as an AGE inhibitor[119].

Pyridoxamine, (form of vitamin B6 that naturally exists) and benfotiamine (a lipid-soluble derivative of thiamine), are known to inhibit AGE formation. In phase 2 trials using pyridoxamine in diabetic patients suffering from obvious diabetic nephropathy, a significant decline in the serum levels of creatinine from baseline was observed although this was not accompanied by a corresponding change in urinary albumin excretion[120]. Using benfotiamine in type 2 diabetic patients prevented both macro- and microvascular endothelial dysfunction and oxidative stress induced by an AGE rich meal[121]. Moreover, the combined use of benfotiamine and alpha-lipoic acid normalized elevated AGE levels and blocked the enhancement hexosamine-modified protein formation in monocytes in type 1 diabetic patients[122].

AGE degradation (AGE cross link breaker, ALT-711)
Cross link breakers such as ALT-711 contain a thiazolium structure that is capable of breaking α-carbonyl compounds by cleaving the carbon-carbon bond between carbonyls. *In vitro*, incubation of AGE crosslinked collagen with ALT-711 promotes collagen digestibility by metalloproteinases (MMP), a phenomenon used as a key sign of an effective cross link-breaker effect[123].

ALT-711 has been used in a number of small clinical studies to investigate the effects of targeting AGE on cardiovascular complications. A phase Ⅱ clinical trial demonstrated that ALT-711 (210 mg/d, 8 wk) reduced the arterial pulse pressure and improved the compliance of large arteries in older patients (> 50 years) who exhibited age-dependent stiffening of their large arteries secondary to isolated systolic hypertension[124].

Two more phase 2b clinical trial trials currently being undertaken to assess the safety as well as the efficacy of ALT-711 use in the treatment of isolated systolic hypertension with or without left ventricular hypertrophy (LVH) in elderly patients (> 50 years), termed systolic and pulse pressure hemodynamic improvement by restoring elasticity (SAPPHIRE) and systolic hypertension interaction with left ventricular remodeling (SILVER). SAPPHIRE is designed to use multiple doses (four) of ALT-711 over a period of 6 mo in patients with systolic hypertension without LVH whilst the control group will be treated with placebo. The SILVER study is being conducted on patients with systolic hypertension and LVH, including both diabetics and non-diabetics treated with a single dose of ALT-711 and a placebo group for 6 mo. The primary endpoints of both studies are alterations in both systolic blood pressure and pulse pressure. Secondary endpoints involve extra measurements of arterial blood pressure and modifications in definite urological parameters. The results of both trials are still not yet available[125].

In addition, two small and open label clinical trials suggested an important role of AGEs in the development of cardiac dysfunction and HF. In both trials HF patients were treated with an AGE cross-link breaker Alagebrium (ALT-711). In the Distensibility Improvement and Remodeling in Diastolic Heart Failure (DIAMOND) trial, 23 patients with stable diastolic HF received ALT-711 for 16 wk after which patients were assessed by magnetic resonance imaging and tissue doppler which revealed both a reduction of left ventricular mass and an improvement of left ventricular diastolic function[126]. The Patients with Impaired Ejection Fraction and Diastolic Dysfunction: Efficacy and Safety Trial of Alagebrium trial was conducted on 22 patients suffering from systolic HF and diastolic dysfunction who were treated by ALT-711 (35-420 mg). The results of this trial were in line with those of the DIAMOND trial. Doppler assessment showed improvement in isovolumetric relaxation time and consequently reduction in the left atrial pressure. Furthermore, reduced left ventricular mass and left ventricular end-diastolic volume was also reported in some patients[127].

However, a more recent clinical trial BENEFICIAL, a prospective, randomized, double-blind, phase Ⅱ, placebo controlled trial on the effects of the AGE-breaker alagebrium on exercise capacity and diastolic function in 102 patients with systolic HF did not support the findings of the earlier trials. In BENEFICIAL study, patients were assessed after receiving 200 mg alagebrium twice daily or placebo treatment for a period of 36 wk. No improvement was reported in exercise capacity or systolic function of those patients. In addition, no significant change was detectable in a number of other parameters including diastolic dysfunction, NYHA functional class, NT pro-BNP levels and AGE levels (skin autofluorescence). However, the authors highlighted some important points that may explain their findings. Firstly, inclusion into this study did not mandate the presence of diastolic dysfunction as an entry criterion into the trial, and it is this sub group with diastolic dysfunction that may have shown a better response to alagebrium treatment. Secondly, tissue AGE levels were relatively low in the patient cohort, especially since only (18%) of the patients included into this study were diabetic. Finally, the duration of treatment might be to short for alagebrium to produce a therapeutic effect[128].

Anti-RAGE receptor therapies
Recently, therapies targeting the RAGE receptor, e.g., sRAGE have been used in experimental studies and proved to be effective in reducing atherosclerosis in diabetic mice[129,130] as well as microvascular complications of diabetes as diabetic retinopathy[131], nephropathy[132] and neuropathy[98]. The use of the latter in the clinical setting has not yet been approved.

CONCLUSION
Cardiovascular diseases are one of the leading causes of morbidity and mortality in the western world particularly amongst patients with diabetes. AGEs accumulate rapidly in the hyperglycaemic milieu of diabetes and have an important role in the development of the macro and

microvascular complications of diabetes.

Several clinical and experimental studies support the view that AGEs might have a significant role in pathogenesis of heart failure by contributing to its development and progression either *via* indirect mechanisms mediated through enhancing coronary artery disease or directly by inducing myocardial damage independent of vascular effects. AGEs adverse effects on cellular and tissue function arise from their potential to cross link intracellular and extracellular proteins thus altering their function and through binding to their cell surface receptor RAGE activating multiple signalling cascades in many different cells within the cardiovascular system. Preliminary data suggests that targeting AGEs therapeutically may represent a novel treatment strategy in the management of DM and its cardiovascular complications.

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