

Implication of advanced glycation end products (Ages) and their receptor (Rage) on myocardial contractile and mitochondrial functions

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Abstract Advanced glycation end products (AGEs) play an important role for the development and/or progression of cardiovascular diseases, mainly through induction of oxidative stress and inflammation. AGEs are a heterogeneous group of molecules formed by non-enzymatic reaction of reducing sugars with amino acids of proteins, lipids and nucleic acids. AGEs are mainly formed endogenously, while recent studies suggest that diet constitutes an important exogenous source of AGEs. The presence and accumulation of AGEs in various cardiac cell types affect extracellular and intracellular structure and function. AGEs contribute to a variety of microvascular and macrovascular complications through the formation of cross-links between molecules in the basement membrane of the extracellular matrix and by engaging the receptor for advanced glycation end products (RAGE). Activation of RAGE by AGEs causes up regulation of the transcription factor nuclear factor- κ B and its target genes. of the RAGE engagement stimulates oxidative stress, evokes inflammatory and fibrotic reactions, which all contribute to the development and progression of devastating cardiovascular disorders. This review discusses potential targets of glycation in cardiac cells, and underlying mechanisms that lead to heart failure with

special interest on AGE-induced mitochondrial dysfunction in the myocardium.

Keywords Advanced glycation end products · Cardiovascular · Mitochondria · Signaling pathways

Introduction

Advanced glycation end-products (AGEs) were first identified in cooked food as end products from a non-enzymatic reaction between sugars and proteins called the Maillard reaction [1]. Since the discovery that this reaction also occurs *in vivo*, it has been suggested that AGEs may play a role in the pathophysiology of several different diseases [2–5]. In the heart, AGEs have been implicated in the development of myocardial damage via distinct pathways, which include tissue accumulation and interactions with several membrane receptors [2, 6, 7]. The AGE-receptor system could be broadly divided into two arms: one is associated with increased oxidative stress, growth, and inflammatory effects, best represented by the receptor for AGE (RAGE); and the other, involved in AGE detoxification, includes scavenger receptors class A, type II (MSR-AII), and class B, type I (MSR-BI, CD36), as well as AGE receptors 1, 2, and 3 (AGE-R1, -R2, and -R3). The key to RAGE biology is its ability to bind and to transduce the molecular effects of multiple ligands including AGEs S100/calgranulin family, high mobility group box-1 (HMGB-1), cluster of differentiation (CD)11b, amyloid- α peptide and β -sheet fibrils. RAGE regulates a number of cell processes of crucial importance such as inflammation, apoptosis, ROS signaling, proliferation and autophagy [2] (Figs. 1 and 2).

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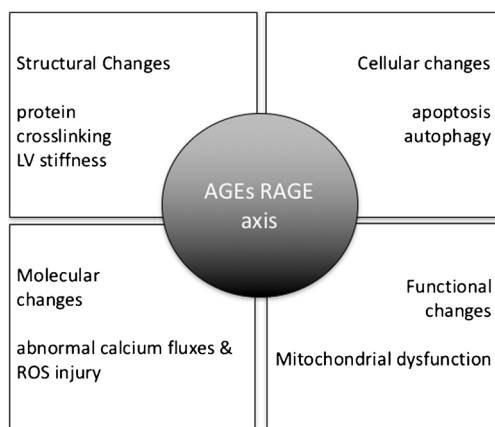


Fig. 1 The multifactorial changes affecting the myocardium at structural, cellular, molecular and functional levels in response to AGE – RAGE axis activation

AGE/RAGE axis in the myocardium

RAGE and cardiac diseases

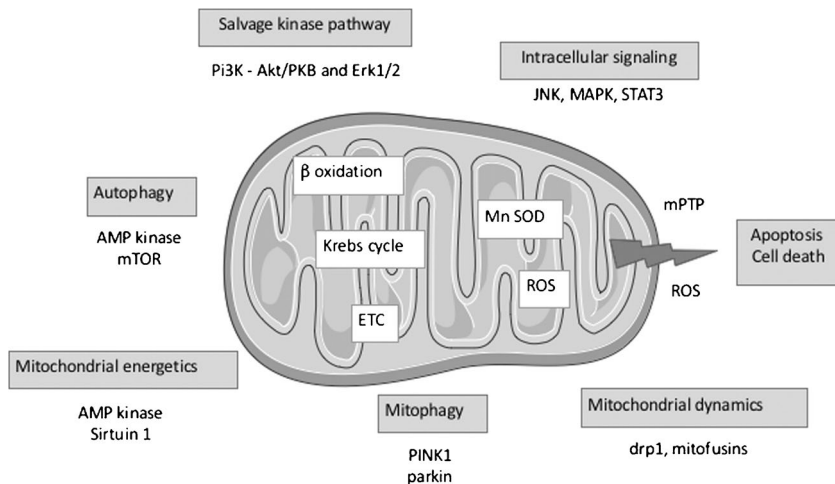
RAGE, a member of the immunoglobulin superfamily of cell surface molecules, is expressed in the heart [6]. RAGE expression increase in the myocardium is associated with reduced heart function in aging and many cardiovascular disorders. Deleterious implication of increased RAGE expression on cardiovascular function is further supported by cardioprotective effects of RAGE blockade or deletion in murine models of atherosclerosis and cardiovascular inflammation [4, 6, 7]. In experimental studies, critical role of RAGE signaling has been investigated in cardiac ischemia reperfusion injury and diabetic cardiomyopathy and attributed to accelerated atherosclerotic disease [8]. In *in vivo* models of myocardial infarction, deficiency of RAGE appears highly cardioprotective against ischemia–reperfusion injury [9–11]. In rodent diabetic cardiomyopathy, RAGE blockade also prevents the development of increased left ventricular diastolic

chamber stiffness and damps down cardiac systolic dysfunction [12]. In *ex vivo* isolated perfused heart models, administration of soluble-RAGE (sRAGE) or genetic deletion of RAGE results in decreased cell damage induced by global ischemia–reperfusion [9, 10]. Mechanisms of cardioprotection are related to reduced apoptosis and prevention of adverse signaling pathway activation, such as phosphorylation of c-Jun N-terminal kinase (JNK) and STAT5, transforming growth factor- β 1, suppression of glycogen synthase kinase (GSK)-3 β ser-9 phosphorylation, and activation of nuclear factor (NF)- κ B [5, 13].

Soluble RAGE (sRAGE) as a marker of cardiovascular diseases

In addition to the membrane-bound form of full-length RAGE, the extracellular domain on its own as soluble RAGE (sRAGE) was detected in the extracellular space and blood circulation [7, 14, 15]. This extracellular domain of RAGE only consists of an N-terminal signal peptide and three immunoglobulin domains, one V-type and two C-type domains. sRAGE can derive from either a proteolytic cleavage of the extracellular part of the transmembrane receptor, called cleaved (cRAGE) or alternative splicing of RAGE [14–16]. Matrix metalloproteases were shown to be involved in ectodomain shedding of sRAGE. This form of sRAGE, by virtue of its derivation from the full length form of RAGE, is composed of the V–C1–C2 domains. Alternative splicing can lead to altered extracellular domain variants, mostly around the V-domain important for ligand binding, or either to soluble forms of which RAGE v1 (RAGE splice variant 1) formerly called esRAGE (endogenous secretory RAGE), or simply sRAGE, was shown to be the only secreted form [14–16]. Alterations in the reading frame during alternative splicing with introduction of 16 new amino acids allow for specific antibody-based measurement of RAGE_v1/esRAGE. Generally, of the total sRAGE species, esRAGE comprises

Fig. 2 Proposed mechanisms of AGE-induced mitochondrial dysfunction in the myocardium. Asterisks * represent direct AGE(carboxymethyl lysine) targets. ETC: electron transport chain; mPTP: mitochondrial permeability transition pore



about 20 % [14–16]. Many published studies have examined levels of sRAGE and/or esRAGE in multiple distinct populations in health and in disorders in which RAGE ligands are known to accumulate [14]. Soluble RAGE may exert its benefit by sequestering RAGE ligands and blocking their interaction not only with RAGE, but, perhaps, with other cell surface receptors.

Many studies have also examined the relationship between sRAGE/esRAGE levels and the status of cardiovascular diseases [17]. For example, RAGE is over-expressed in atherosclerotic lesions and its activation has been implicated in the onset and progress of cardiovascular complications. In ApoE $-/-$ mice, which spontaneously develop atherosclerotic lesions, sRAGE treatment prevents plaque formation [18]. Nevertheless, sRAGE levels in humans do not follow the logical thought of a protective decoy receptor. In patients with hypertension, sRAGE has been positively associated with inflammation and disease [19, 20], but also found decreased in comparison to normotensive subjects [21]. In non-diabetic patients with coronary artery disease (CAD) sRAGE levels are decreased [22, 23], although Mulder and colleagues did observe a positive correlation between sRAGE and skin autofluorescence [24]. It was further found lower esRAGE levels in CAD patients in both type 2 diabetic and non-diabetic populations although total sRAGE levels were increased in diabetic patients with CAD. A positive association of sRAGE with chronic heart failure and atrial fibrillation has been observed [25–27]. In patients with kidney dysfunction, who usually present increased levels of sRAGE, negative correlations have been observed for atherosclerosis, vascular calcification and ventricular hypertrophy [28–30]. A recent meta-analysis, investigating the association of RAGE polymorphisms and sRAGE with coronary artery disease, concluded that sRAGE levels are not significantly associated with CAD, but a positive association is observed in studies with diabetic patients. More recent studies demonstrated that sRAGE levels are elevated in relation to acute coronary syndrome, however during a very narrow time period, indicating that the time of sampling needs attention [30, 31]. Several investigators have reported that serum levels of sRAGE are lower in patients with stable coronary artery disease and atherosclerotic burden disease in nondiabetic men [30, 31]. In summary, conditions where sRAGE or esRAGE levels are elevated include type 2 diabetic patients with coronary artery disease or with atherosclerotic burden, whereas serum levels of sRAGE are lower in patients with coronary artery disease and atherosclerotic burden disease in nondiabetic men.

Hence, in human heart diseases, measurements of total plasma levels of sRAGE are associated with important cardiovascular outcomes, but the directions of associations have varied. Some studies have demonstrated an association between higher sRAGE levels and incident cardiovascular disease and mortality [31], whereas others have reported a

significant inverse association between sRAGE and cardiovascular disease and all-cause mortality [32].

Endogenous AGE accumulation in the myocardium

AGEs accumulate in the human body with age and cardiovascular deposition of AGEs is accelerated in several pathological conditions, such as chronic heart failure and diabetic cardiomyopathy [8]. Accumulation of AGEs is implicated in the development of myocardial dysfunction via two distinct pathways.

Firstly, AGEs cause multiple myocardial changes via interaction with RAGE [2, 6, 13]. Localization of RAGE expression in the myocardium predominates in vascular endothelium and capillaries [33]. RAGE expression is also reported in cardiomyocytes [34, 35] and consistent *in vitro* and *in vivo* studies demonstrated the important role of AGE-RAGE interactions for the development of reduced diastolic and systolic performance [13]. Secondly, endogenously produced AGEs are consistently implicated in the development of cardiac dysfunction in several animal models [36–38]. AGEs accumulate in the myocardial interstitium in between cardiomyocytes [34], which may affect physiological properties of proteins in the extracellular matrix of cardiac cells. In such experimental studies, beneficial effect of various AGE-lowering strategies confirms the role for AGEs in heart stiffness and further development of cardiomyopathy. In heart of diabetic rodents, immunoassay detection of N-epsilon carboxymethyl lysine (CML) shows its deposition in endothelial and smooth muscle cells of the microvasculature [9]. In humans, abundant microvascular AGEs deposition is observed in patients with diabetic cardiomyopathy [39]. Deposition of AGEs is also detected within cardiomyocytes in tissue specimens of patients undergoing epicardial biopsy or heart transplantation [35, 40]. In these studies, diffuse positive CML immunostaining may be detected in the heart whether or not patients had type 2 diabetes. Immuno-electron microscopic methods further confirm existence of intracellular anti-AGE binding sites. These sites of AGE binding are widely distributed in cardiomyocytes, and preferentially in myofibril sections and within mitochondria. Whereas only limited human studies have been published, direct role of AGEs in the development of cardiac dysfunction has been investigated extensively in animals.

A number of drugs had been developed to interfere with the glycation pathway. Despite most of them are solely used in preclinical settings, some are approved for human treatment [41, 42]. Mechanisms of action of AGE inhibitors and breakers include post-Amadori inhibition due to dicarbonyl intermediates trapping (aminoguanidine, pyridoxamine) and breaks of carbon-carbon bonds between carbonyl (alagebrium) [41, 42]. The effects of aminoguanidine on diabetes-related complications and/or measures of glycation have been investigated in two large double-blind RCTs

involving individuals with type 1 and type 2 diabetes mellitus, which were terminated early due to unfavourable perceived risk-to-benefit ratio [43]. Alagebrium has been extensively investigated in uncontrolled clinical trials with some cardiovascular beneficial effects [41, 42]. One RCT on alagebrium showed beneficial effects on a range of cardiovascular variables in hypertensive individuals [44], whereas another in heart failure patients did not [45]. In addition, in uncontrolled (single-arm) studies in individuals with hypertension [46] and diastolic heart failure [47], improvements were observed in aortic augmentation index (as a marker of pulse wave reflection and arterial stiffness) and flow-mediated dilation (as a marker of endothelial function) after 8 weeks and in left ventricular mass and diastolic function after 16 weeks of alagebrium treatment. Hence, short-term use of AGE cross-link breakers such as alagebrium hydrochloride yielded favorable results in uncontrolled trials, yet failed in the large outcome trial BENEFICIAL [48]. The apparent lack of benefits in these trials may be related to small sample size, short term use of alagebrium and short follow-up period regarding decades of accumulation of AGEs long-life cardiac proteins. These limitations have been recently challenged in a study [49] that enrolled 62 healthy older individuals with sedentary life, who were randomized into four groups; training + alagebrium, training + placebo, no training + alagebrium and no training + placebo. After one year of treatment, alagebrium had no effect on hemodynamics, left ventricular shape or exercise capacity, but improved left ventricular stiffness. Overall, available results suggest that short-term use of alagebrium might not be sufficient to alter LV diastolic function and aerobic capacity, whereas long-term alagebrium use did show modestly favorable effects on age-associated LV stiffening. This favorable effect of alagebrium on LV stiffness was most prominent in individuals with combined alagebrium and exercise training [49].

Effects of dietary AGEs on myocardial function

Apart from endogenous AGE formation, AGEs and their precursors are also absorbed by the body from exogenous sources through consumption of highly heated processed foods [1]. Large databases of different food items and their AGE contents have been published [50]. Beside carboxymethyl lysine CML, a broad range of other AGEs have been detected in food [1]. Human studies revealed that approximately 10 % of orally administered AGEs are absorbed, two-thirds of the which remain in the body and only one-third is excreted into urine [51]. Consumption of AGE-rich meal (3-fold higher AGE content) elicits increase serum AGE concentration by about 1.5. Available information regarding distribution and metabolism of AGEs in human is scarce, yet AGE distribution after ingestion is associated with temporary liver accumulation [52]. Accumulation of dietary AGEs in organs induce structural

deformation or cross-linking of proteins and interaction with AGE receptors. Comparisons of AGE-rich diets with low AGE diets have been investigated in human studies [53], which suggest implication of dietary AGEs in the pathogenesis of various cardiovascular disorders [54–56]. For example, lowering of dietary AGEs reduces neointimal formation after arterial injury in genetically hypercholesterolemic mice [57]. Likewise, low AGE diet for 2 months in diabetic apolipoprotein E-deficient mice can reduce serum AGE levels, tissue AGE and RAGE expression, as well as inflammatory cell infiltration and tissue expression of tissue factor, vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 [58]. Our group has shown that carboxymethyl lysine CML-enriched diet-fed mice display impaired endothelium-dependent relaxation, which is associated with increased expression of RAGE and VCAM-1 in the aortic wall [55]. RAGE-deficient mice are protected against CML-rich diet-induced endothelial dysfunction. In line, chronic vein injections with advanced oxidation protein products elicit in rats impairment of endothelium-dependent relaxation, which was accompanied by decreases in serum nitric oxide and activities of glutathione peroxidase and superoxide dismutase [59]. Interestingly, these experimental data are consistent with clinical studies in diabetic patients, in which lowering AGE content in meal elicits significant reduction of stress oxidant and pro-inflammatory biomarkers [56]. In addition, endothelial function evaluated by flow-mediated dilation was acutely (4–6 h) impaired following high-AGE meal, which was paralleled by an impairment of microvascular function and increased levels of serum AGEs and markers of endothelial dysfunction and oxidative stress in patients with type 2 diabetes mellitus [56]. Of note, these results are not universal in the literature, as 6-wk. consumption of AGE-rich diet in healthy adults had no significant impact on endothelial function and inflammatory mediators [60, 61]. Overall, these findings claim for critical involvement of food-derived AGEs in the development of vascular inflammation and endothelial dysfunction, which represent the initial steps leading to cardiovascular diseases in humans.

In heart and vessels, alterations of collagens and elastin induced by AGE-modifications may contribute to decreased elastic properties, thereby playing a role in arterial and cardiac stiffness. First, CML-rich diet is responsible for heart AGE accumulation. Second, oral administration of methylglyoxal in rats increases cardiovascular collagen content and facilitates cardiac perivascular adventitial fibrosis [62]. In this study, methylglyoxal was orally administered to Dahl salt-sensitive rats with a low-salt diet. Progressive increase in systolic blood pressure was observed after 12 weeks of methylglyoxal administration, which was associated with increased inflammation and carbonyl and oxidative stress. Methylglyoxal also induced increases in total myocardial collagen content and perivascular adventitial fibrosis scores in Dahl salt-sensitive

rats that was attributed at least in part to renine-angiotensin-mediated pathway. Intraperitoneal and subcutaneous chronic administration of methylglyoxal in rodents induces biochemical and molecular abnormalities characteristic of type 2 diabetes and provokes diabetes-like microvascular damage. Of major interest, oral chronic administration of methylglyoxal to normal rodents can impair survival pathways to heart ischemia-reperfusion injury and mimic many alterations found in the diabetic cardiomyopathy [63]. Rats were submitted to methylglyoxal administration in water for 14 weeks, which induced increased levels of cardiac carbonyl protein and superoxide anion. Oral methylglyoxal impaired survival protein response to ischemia reperfusion injury in isolated hearts, as evidenced by increased caspase-3 expression and reduced Akt phosphorylation and Bcl2/Bax ratio [63].

Hence, AGE-rich diet and high AGE content in food may impact the diabetic myocardium facilitating pro-inflammatory and pro-oxidative cardiovascular states as well as heart fibrosis. Evidence presented in systematic reviews support that high AGE content in food is a risk factor of cardiovascular diseases in humans through increased inflammation and oxidative stress processes [53, 64]. Meta-analyses of long-term trials of dietary AGE restriction provide evidence favoring reduction in oxidative stress and pro-inflammatory biomarkers in healthy adults and diabetic patients.

Mechanisms of AGE/RAGE axis-induced myocardial dysfunction

Diastolic dysfunction is a key feature of myocardial dysfunction in aging and the diabetic heart. Mechanisms of diastolic dysfunction include decreased ventricular relaxation, abnormal active relaxation, and/or an increase in ventricular and arterial stiffness. Several studies thoroughly investigated the role of advanced glycation end products (AGEs) in the background of diastolic dysfunction both in experimental and clinical studies [37, 38, 65].

AGE-induced myocardial wall stiffness

First, AGE may occur in the extracellular compartments, as previously mentioned. Accumulation of AGEs in the extracellular milieu causes excessive cross-linking of extracellular matrix proteins, which increases cardiac muscle rigidity, thereby causing diastolic dysfunction [37, 38, 65–67]. Also, AGE depositions perturb cell-matrix interactions, alter cell adhesion, and increase vascular permeability, which may all impair cardiac wall kinetics. At the intracellular level, AGE deposition can indirectly augment diastolic left ventricle (LV) stiffness through reduced nitric oxide bioavailability [68]. Several mechanisms by which AGEs reduce or block NO activity have been proposed [68–71]. For example, AGEs are able to

quench and inactivate endothelium-derived NO via a direct chemical reaction. AGEs may also reduce the half-life of endothelial NO synthase (eNOS) mRNA through an increased rate of mRNA degradation and reduced eNOS activity. Another mechanism proposes that AGEs impair NO production via the binding of CML residues to endothelial AGE receptors, causing a reduction in phosphorylation of serine residues in eNOS, resulting in deactivation of the enzyme [72]. In these investigations, *in vitro* AGE exposure decreased in eNOS activity related to changes in phosphorylation of ser1179 in the eNOS enzyme, which prevented anti-CML or anti-AGE-R1 antibodies. Overall, AGE accumulation reduces endothelially-produced nitric oxide, which in turn lowers myocardial nitric oxide bioavailability leading to diastolic dysfunction. This is supported by observations showing that AGE cross-link breakers may improve flow-mediated dilation of the brachial artery [46] and because blunted coronary endothelial nitric oxide release typically worsens diastolic LV function [73].

Second, AGEs interact with the receptor RAGE, activation of which cause increased fibrosis signaling via up-regulation of transforming growth factor- β (TGF- β) [6, 7]. For example, AGE/RAGE signaling results in increased synthesis of various types of collagen, laminin, and fibronectin in the extracellular matrix, most likely via up-regulation of TGF- β intermediates [5]. In addition, activation of downstream signaling kinases such as p38, extracellular signal-regulated kinase 1/2 (ERK 1/2), nuclear factor- κ B (NF- κ B), and c-Jun N-terminal kinase (JNK), have been shown to mobilize multiple transcription factors to stimulate expression of growth factors and extra-cellular matrix protein accumulation [74]. It has been proposed that multiple outcomes of AGE/RAGE signaling operate through protein kinase C (PKC), activation of which is followed by increased extra-cellular matrix protein expression [75].

AGE/RAGE axis disrupt intracellular calcium handling

Interactions of AGE/RAGE have been suggested to impair cardiac Ca^{2+} handling by reducing both systolic and diastolic intracellular Ca^{2+} concentration and prolonging the decay time of Ca^{2+} transient [76]. Indeed, AGEs – RAGE interactions elicits a significant delay in Ca^{2+} reuptake in cardiomyocytes. As a consequence, the duration of repolarization phase of cardiac contraction is increased, which eventually causes diastolic dysfunction. In addition, RAGE overexpression per se results in Ca^{2+} transient decay prolongation that may be further impaired after AGE incubation of cardiomyocytes [76]. In these studies, AGE / RAGE interactions have no effects on protein expression levels of sarcoplasmic reticulum SR Ca^{2+} -ATPase (SERCA2), phospholamban, and $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) [76]. Alternatively, cross-linked AGEs on long-lived intracellular cardiac sarcoplasmic

reticulum (SR) proteins such as SERCA2a and ryanodine receptor (RyR2) may be responsible for observed deleterious effects of AGEs in cardiomyocytes [77]. In line, AGE / RAGE interactions perturb SR homeostasis leading to RyR2 abnormal activity and Ca^{2+} spark frequency increases, which may lead to SR Ca^{2+} leak [77]. In turn, enhanced Ca^{2+} spark-mediated SR Ca^{2+} leak can partially deplete SR Ca^{2+} content, resulting in decreases of systolic Ca^{2+} and contractile force transients. These results indicate that post-translational modifications of the SR proteins by AGEs could lead to an alteration in calcium homeostasis leading to abnormal cardiac cell relaxation and systolic dysfunction [78]. Recent findings suggest that AGEs incubation of cardiomyocytes also elicits marked depression in sarcolemma Na^+/K^+ ATPase activity that appears to play a major role in the development of intracellular calcium overload and abnormal cardiac relaxation [79].

AGE-RAGE axis in the pathogenesis of cardiac oxidative stress

It is now well accepted that AGE/RAGE engagement triggers the generation of ROS, and subsequent activation of a complex cascade of signal transduction events, as demonstrated for p21 ras, p44/p42 mitogen-activated protein kinases, and nuclear factor kappa-B NF- κ B [13, 80]. In human cardiomyocytes, growing evidence indicates that AGEs have deleterious effects, as AGEs may trigger reactive oxygen species (ROS) generation, apoptosis, MAPK activation and nuclear O-GlcNAcylation [81]. As previously mentioned, AGEs also reduce eNOS activity, which may contribute to the onset of ROS production through NOS uncoupling mechanism. In addition, when AGEs interact with RAGE, they activate NF- κ B, nuclear translocation of which increases gene expression of many cytokines, adhesion molecules and activation of NADPH-oxidase and ROS generation [73, 80, 82]. The primary mechanism by which RAGE generates oxidative stress is via activation of NADPH oxidase and amplification mechanisms in the mitochondria may further drive ROS production. Hence, the contribution of AGEs should be considered as a relevant mechanism triggering ROS production and oxidative stress in the heart [82].

In the myocardium, AGEs have been consistently shown to increase oxidative stress in many cell types. In cultured cardiomyoblasts exposed to AGEs, ROS production increases in a time and dose dependent manner, whereas maintenance of the antioxidant activity of antioxidant enzymes, such as glutathione peroxidase, is lost [4, 83]. Many studies support that AGE-RAGE-induced cytosolic ROS production can facilitate mitochondrial superoxide production in hyperglycemic environments. Of note, demonstration of mitochondria as the specific source of ROS in response to AGEs in cardiomyocytes is scarce, whereas production of ROS in cardiac endothelial cells,

vascular smooth muscle cells and inflammatory cells has been consistently described [83].

AGE-RAGE axis interactions with apoptosis / autophagy pathways

Previous studies have demonstrated that AGEs alters endothelial F-actin cytoskeleton morphology and increased endothelial cell monolayer permeability through activation of p38MAPK and RhoA kinases [3]. These kinases then phosphorylate threonine residues of moesin and ezrin/radixin/moesin (ERM) proteins and lead to the linking of activated ERM with F-actin, resulting in cytoskeleton reorganization and disruption of endothelial barrier function [84]. In cardiomyocytes, AGEs also disrupt intracellular extra-sarcomeric cytoskeleton by specifically targeting desmin network anchorage at the Z-disk of the sarcomere [85]. In these experiments, AGEs alter the striated organization of desmin, but not actin, which was attributed to α B crystallin phosphorylation. These observations are crucial because desmin filaments interconnect myofibrils and mitochondria and mutations of desmin or α B crystallin have been directly involved in the development of dilated cardiomyopathy in humans. In addition, cytoskeleton disruption possibly due to cytoskeletal reorganization or depolymerization can increase ROS generation [86].

The apoptotic signal exerted from RAGE can be mediated in cardiomyocytes through stimulation of p38MAPK and JNK pathways [3]. Both death-receptor and mitochondrial pathways are activated in response to RAGE expression with increased expression of proapoptotic caspase-3 and caspase-9, and decreased expression of antiapoptotic Bcl-2 [3, 87]. In contrast, RAGE deficient cardiac cells are more resistant to cell apoptosis induced by myocardial ischemia–reperfusion. In these studies, RAGE deficient cells have elevated levels of Bcl-xL, decrease in cytochrome c release, and reductions in phosphorylation of JNK and STAT5 [88]. *In vivo*, RAGE deletion and sRAGE administration are also protective against ischemia–reperfusion injury, which is related to increased phosphorylation levels of JNK, STAT3 and STAT5 [89, 90]. Pro-apoptotic pathway p53–Bcl-2-associated X protein is also implicated in the regulation of apoptosis by RAGE. For example, increases in p53 phosphorylation and activation have been observed in S100B-treated cardiomyocytes in the border infarct zone [90].

While biological functions of sRAGE are not clearly defined, sRAGE can significantly inhibit mitochondrial cytochrome c release and caspase-3 and caspase-9 activities as well as decrease of Bax to Bcl-2 ratio following heart ischemia–reperfusion [89, 90]. Studies in cardiomyocytes and heart mice show that RAGE inhibition can influence myocardial cell fate and heart energy metabolism following ischemia–reperfusion [9, 10]. These results support that accumulation of AGEs and increased RAGE expression can elicit

mitochondrial dysfunction. For example, at the end of ischemia–reperfusion, levels of myocardial ATP are higher in RAGE-null mice than in littermates [9]. Further experiments in diabetic mice using RNA interference demonstrate that AGE-induced cardiomyocyte dysfunction is related to dissipation of the mitochondrial membrane potential, *i.e.*, $\Delta\psi_m$ depolarization, and reduced GSK-3 β inactivation [89, 90]. In these studies, sRAGE administration can prevent $\Delta\psi_m$ depolarization, delay mitochondrial transition pore opening, thus improving mitochondrial metabolism.

Autophagy is a catabolic process in which cytoplasmic proteins or whole organelles are sequestered and degraded by autolysosomes [87]. Inhibition of mammalian target of rapamycin (mTOR) complex 1 (mTORC1) plays a major role in the induction of autophagy, whereas mTORC1 activity is inhibited in RAGE deficient cells [91]. Another important positive regulator of autophagosome formation is the adenosine 5'-monophosphate-activated protein kinase (AMPK), activation of which is also RAGE-dependent [87]. Phosphatidylethanolamine-conjugated LC3-II localized in the membrane of autophagosome and p62/sequestosome 1 SQSTM1 that binds LC3 and recruits proteins into autophagosomes for degradation, are considered as major autophagy markers. In cultured cardiomyocytes, incubation with AGEs is associated with increased levels of autophagy-associated protein LC3-II and down regulation of SQSTM1/p62, whereas the number of autophagosomes is increased [92, 93]. Interestingly, apoptotic cardiac cell death triggered by AGEs may be limited by simultaneous autophagy activation. In these *in vitro* experiments, PI3K/AKT/mTOR and ERK signaling pathways have implicated in autophagy induced by AGEs [92].

AGE-RAGE axis perturbs mitochondrial function and signalization

AGE-RAGE axis and mitochondrial energetics

Growing evidence suggest that AGEs induce deleterious effects on mitochondrial respiration and oxidative phosphorylation. As state above, cardiac ATP levels are higher in RAGE-null mice than in littermates in response to ischemia–reperfusion. Likewise, incubation of AGEs in different cell lines promotes $\Delta\psi_m$ dissipation and decrease of intracellular ATP levels [9, 89, 90]. Oxygraphy studies in cardiomyocytes have further shown reductions of respiratory control ratio and activity of respiratory complexes in AGEs treated cells [94]. Interestingly, expression of several mitochondrial proteins is reduced in response to AGE accumulation with aging [95, 96]. Increased glycative damage is found in many of these proteins, which are identified as crucial respiratory chain complex I and IV components and key enzymes implicated in fatty acid

β -oxidation and tricarboxylic cycle [96]. This current evidence suggest that reduced capacity for fatty acid oxidation can be induced by AGE accumulation, which represent a key step in the development of insulin resistance. In addition, high level of glycation has been demonstrated for catalase in aging [95]. Glycation of catalase is responsible for decrease in its activity that may compromise the overall antioxidant enzyme defense system [95, 96]. Catalase inactivation in aging would lead to oxidative damage not only directly through H₂O₂ and its derivative, but also indirectly through inhibition of superoxide dismutase SOD, leading in turn to increased levels of superoxide radicals.

Two important mediators implicated in cell metabolism might be silent mating type information regulation 2 homolog 1 (SIRT1), a NAD(+)-dependent deacetylase, and AMP-activated protein kinase (AMPK), an energy-sensing kinase. Sirtuins such as SIRT1 are conserved protein NAD(+)-dependent deacetylases and thus their function is intrinsically linked to cellular metabolism. The versatile functions of sirtuins including, more specifically, SIRT1 are supported by their diverse cellular location allowing cells to sense changes in energy levels in the nucleus, cytoplasm, and mitochondrion. SIRT1 plays a critical role in metabolic health by deacetylating many target proteins in numerous tissues, including the myocardium. Evidences are mounting that both SIRT1 and AMPK play important roles in AGEs induced cellular metabolism deficit [1, 3]. For example, high levels of serum AGE markers, such as carboxymethyl lysine and methylglyoxal, are strongly linked to suppressed levels of SIRT1 [56]. Vascular AGEs accumulation in CML fed mice is associated with reductions in both SIRT1 mRNA and protein expression [55]. Exposure of mice to methylglyoxal-enriched diet can result in severe SIRT1 deficiency, shift to a pro-oxidative and inflammatory phenotype, and early insulin resistance [97, 98]. Proposed molecular mechanisms linking AGE–RAGE axis and SIRT1 included ubiquitination of SIRT1 and its further degradation via the proteasome pathway [99]. These findings are crucial regarding the critical role of SIRT1 that regulates important metabolic and physiologic processes including stress resistance, metabolism, apoptosis, autophagy and energy balance. The role of SIRT1 and AMPK in AGE-induced myocardial dysfunction and cardiac metabolism deficit has to been demonstrated.

AGE-RAGE axis and mitochondrial dynamics

In non-cardiac cells, mitochondria are highly mobile and dynamic organelles that continuously fuse and divide. The fission and fusion processes are essential for preserving the stability of mitochondrial contents and the integrity of mtDNA. Mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and optic atrophy 1 (OPA1) regulate the fusion of mitochondria. Mitochondria may also undergo rapid fragmentation via fission protein dynamin-related protein 1 (Drp1) with a concomitant increase

in ROS formation after exposure to high glucose levels [100]. In this context, recent *in vitro* studies suggest unbalanced mitochondrial dynamics in the presence of CML-BSA. CML-BSA incubation is associated with increased expression of the fission protein Drp1 and decreased expression of the fusion proteins Mfn1 and Mfn2 [101]. Overall these results suggest that AGEs may disrupt mitochondrial network dynamics and trigger mitochondria fragmentation in non-cardiac cell lines.

In the myocardium, unbalancing of mitochondrial dynamics toward the fission along with increase in fission proteins and $\Delta\psi_m$ dissipation indicate poor mitochondrial health [102]. Mitophagy describes degradation of damaged mitochondria through autophagy, which is part of mitochondrial quality control. One of the mechanisms that induce mitophagy in mammalian cells is the accumulation of PTEN-induced putative kinase 1 (PINK1) upon mitochondrial depolarization. PINK1 then recruits Parkin, an E3 ubiquitin ligase, to the mitochondrial outer membrane [102]. PINK1-dependent recruitment of Parkin may induce mitophagy through processes involving ubiquitination of outer membrane proteins that mark the organelle for autophagosomal engulfment. Notably, AGEs can induce autophagy process through PI3K/AKT/mTOR and ERK signaling pathways [92, 93]. In line, recent studies in cardiac cells revealed that AGEs accumulation increases expression of Beclin-1 and LC3 II/I, and increase the number of autophagic vacuoles [92]. Moreover, the levels of both PINK1 and Parkin are increased with CML-BSA treatment in non-cardiac cell lines [101, 103]. These preliminary observations suggest that AGE accumulation can trigger mitophagy in response to mitochondria damage. One hypothesis would be thus that AGEs induce damage to mitochondria and their degradation by mitophagy, leading to cardiac cell dysfunction.

Conclusion

Although many of the deleterious effects of AGEs on the cardiovascular system have been described in the vasculature, the negative impact of AGE-RAGE axis activation on myocardial contractile-function and metabolism has been described in both experiment and clinical myocardial disorders. Proposed mechanisms of these deleterious effects include triggering of cardiac pro-inflammatory and pro-oxidative states, myocardial fibrosis, intracellular calcium flux and energetics abnormalities.

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