Vascular effects of advanced glycation endproducts: Clinical effects and molecular mechanisms*

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ABSTRACT

The enhanced generation and accumulation of advanced glycation endproducts (AGEs) have been linked to increased risk for macrovascular and microvascular complications associated with diabetes mellitus. AGEs result from the nonenzymatic reaction of reducing sugars with proteins, lipids, and nucleic acids, potentially altering their function by disrupting molecular conformation, promoting cross-linking, altering enzyme activity, reducing their clearance, and impairing receptor recognition. AGEs may also activate specific receptors, like the receptor for AGEs (RAGE), which is present on the surface of all cells relevant to atherosclerotic processes, triggering oxidative stress, inflammation and apoptosis. Understanding the pathogenic mechanisms of AGEs is paramount to develop strategies against diabetic and cardiovascular complications.

Keywords Advanced glycation endproducts; Endothelium; Vascular

1. INTRODUCTION

Morbidity and mortality among people with diabetes mellitus are mostly triggered by premature cardiovascular disease (CVD) [1,2]. Elevated levels of circulating advanced glycation endproducts (AGEs) in the presence of hyperglycemia are believed to play a major role in the pathogenesis of macrovascular and microvascular disease observed in diabetes mellitus [3–6]. This review briefly describes the source of AGEs, their mechanisms of action and the specific effects on cells implicated in vascular homeostasis. Furthermore, key data from animal studies is presented along with the clinical evidence for their effects at the macrovascular and microvascular levels.

1.1. Literature research

A PubMed search was performed until September 2013 using the terms “advanced glycation endproducts” in combination with the terms “microvascular”, “macrovascular”, “endothelium”, “retinopathy”, “nephropathy”, “neuropathy” or “cardiovascular”.

2. DEFINITION AND SYNTHESIS OF AGEs

AGEs are a heterogeneous group of compounds formed by the nonenzymatic glycation of proteins, lipids or nucleic acids [7,8] within the so-called “Maillard reaction”, a tribute to the French scientist Louis Camille Maillard (1878–1936). This reaction consists of several steps (Figure 1). The first, reversible step takes place between the carbonyl group of a reducing sugar and an aminoterminal group of a protein, lipid or nucleic acid generating a so-called “Schiff base”. By structural irreversible rearrangements, more stable keto-amines are formed, called Amadori products (e.g. the HbA1c) [9]. The Amadori products undergo further structural changes through oxidation, dehydration and degradation to finally yield highly stable AGEs compounds [9,10].

The carbonyl groups necessary for the reaction do not exclusively originate from carbohydrate metabolism, but can be also formed during lipid or protein degradation [11]. When the carbonyl group originates from the lipid catabolism (e.g. malondialdehyde), the products of the reaction are termed “advanced lipoxidation endproducts” (ALEs) [11]. However, the differentiation between AGEs and ALEs is not always possible: N-carboxymethyllysine (CML), for example, can be formed during either carbohydrate or lipid catabolism and can therefore be attributed both terms AGE or ALE.

Usually the reaction leading to the formation of AGEs may take weeks to years and may affect especially long-lived substrates like collagen [12]. Under certain conditions, such as increased substrate availability (e.g. hyperglycemia), increased temperature and increased oxidative stress, the formation of AGEs can be reduced to several hours [13], also affecting short-lived substrates like hormones (e.g. insulin), enzymes,
amino acids or lipids and thus inducing functional and/or structural changes [14–18]. Highly reactive dicarbonyl compounds are generated during the conversion of Amadori products to AGEs, with methylglyoxal (MG) and 3-deoxyglucosone being the most studied AGE dicarbonyl precursors [19,20]. AGEs act either by modifying substrates, or by interacting with specific receptors; these mechanisms will be detailed in the course of this article.

Three main groups of AGEs have been described: (1) fluorescent cross-linking AGEs (e.g. pentosidine and crossline); (2) non-fluorescent cross-linking AGEs such as imidazolium dilysine cross-links resulting from reactions between glyoxal derivatives and lysine residues; (3) non-cross-linking AGEs (e.g. CML) [21–24].

### 3. SOURCES AND METABOLISM OF AGES

AGEs can be formed within the organism (endogenous source) or can originate from exogenous sources. While AGEs are better known as by-products of hyperglycemia, they also form within food during heat-enhanced cooking [25]. Evidence has accumulated that dietary AGEs are partially absorbed [26,27] and either retained in the body or excreted in the urine [27–29]. These dietary AGEs represent an important source for circulating AGEs under in vivo conditions [30–32]. Moreover, smoking also serves as an additional exogenous source of AGEs [33].

The most important mechanisms involved in the degradation of endogenous AGEs are extracellular proteolysis as well as the AGEs-receptor (AGER1)-mediated intracellular uptake and degradation within cells like tissue macrophages (Figure 3) [8]. The degradation of AGEs by macrophages generates low-molecular, soluble peptides, also known as “second generation AGEs” that leave the cells, appear in blood and are finally excreted by the kidney depending on the kidney function [29,34]. In the liver, Kupffer cells and endothelial cells also seem to play an important role in the endocytosis and degradation of glycated substrates [35]. Under physiologic conditions, AGEs accumulate with age [36], but their accumulation seems to be exacerbated by some pathologic conditions: diabetes mellitus, renal failure, cardiovascular disease, Alzheimer’s disease, rheumatoid arthritis, and others [3,10,31,37–39]. In these conditions, AGEs do not seem to be innocent bystanders, but rather contribute to pathophysiologic alterations.

### 4. METHODS FOR MEASURING AGES

Several methods are available for the measurement of AGEs. Circulating or tissue-bound AGEs can be measured by (i) enzyme-linked immunosorbent assays (ELISA) using monoclonal or polyclonal antibodies [30], (ii) fluorescence spectroscopy using the fluorescence properties of some AGEs [40], or (iii) high-performance liquid chromatography (HPLC) and mass spectrometry (MS), with the latter method being probably the most reliable [10]. MS-based methods allow, for example, for measurements of the AGEs’ representative CML in the urine by combining isotope dilution and gas chromatography (GC)–MS analysis. This method is sensitive, reproducible,
accurate, rather cheap and therefore often used in laboratories for the diagnosis and monitoring of age-related chronic diseases [10,41]. Ahmed et al. [42] have used liquid chromatography (LC) with triple quadrupole MS detection for the quantitative measurement of protein glycation, oxidation and nitration adducts. In patients suffering from uremia, LC has been combined with tandem mass spectrometric (LC-MS/MS) detection to accurately quantify glycation adducts in plasma, urine, and dialysate samples [43]. Finally, matrix-assisted laser desorption ionization-mass spectrometry with time-of-flight detection (MALDI-TOF/MS) seems to be a promising tool to analyze AGE-modified proteins [10,44]. Tissue-bound AGEs are usually measured in the skin because of its easy accessibility. The gold standard is represented by the biochemical analysis of skin biopsies [45], but rapid, non-invasive methods have also been applied to measure skin autofluorescence [40]. The measurement of skin autofluorescence was validated against biochemical analyses of AGEs in dermal tissue in biopsies taken from the same spot on the arm [40,46]. Subsequently, several studies have demonstrated that auto-fluorescence is elevated in people with diabetes [47] and in people with end-stage renal disease [48] when compared to control subjects. Overall, the choice of the method depends on the purpose of the study. For example, rapid, postprandial changes, or changes within short interventional studies, are usually assessed by measuring circulating AGEs rather than tissue-bound AGEs, while epidemiologic studies and long-term interventional studies focus on the measurement of tissue AGEs. For large screening studies, the measurement of skin autofluorescence represents a tempting alternative [49].

5. MECHANISMS MEDIATING THE VASCULAR EFFECTS OF AGEs

Some of the mechanisms mediating the detrimental effects of AGEs on the vasculature are related to inflammation and oxidative stress [50], increased glycation of low-density and high-density lipoproteins (LDL, HDL) [51], activation of the pro-inflammatory inducible nitric oxide (NO)-synthase (iNOS) [52] and decreased NO availability [53]. Further mechanisms comprise the increased production of cytokines, e.g. insulin-like growth factor-1 (IGF-1) or the platelet derived growth factor (PDGF), which modify the migration of monocytes and macrophages as well as the proliferation of vascular smooth muscle cells (VSMC) [54,55]. The AGEs-induced effects can be classified according to their site of action or their receptor dependency. AGE-induced damage can occur to the vasculature, to the vascular cells and to the cells implicated in the vascular homeostasis via at least the following 4 mechanisms [56] (Figure 3):

(i) AGEs modify intracellular proteins, including those involved in the regulation of gene transcription [15].
(ii) Precursors of AGEs leave the cells via diffusion and modify nearby extracellular matrix molecules, subsequently altering the signaling between matrix and cells and ultimately causing cellular dysfunction [57].
(iii) AGEs and their precursors modify circulating proteins in the bloodstream, thereby altering their function.
(iv) Circulating proteins modified by AGEs bind to and activate AGEs receptors, thereby altering the production of inflammatory cytokines and growth factors, which in turn drive cellular and tissue damage [30,54]. Indeed, the activation of certain receptors for AGEs (e.g. RAGE) promotes inflammatory response, mainly by the activation of nuclear factor-κB (NFκB), apoptosis, prothrombotic activity, expression of adhesion molecules, and oxidative stress [58–60] (Figure 2). Furthermore, the AGE–RAGE interaction can activate the inducible nitric oxide synthase (iNOS). The iNOS resides mainly in inflammatory cells, is regulated by inflammatory cytokines and can be stimulated by oxidative stress in an NFκB-dependent manner, resulting in toxic concentrations of nitric oxide (NO) [52,61]. The latter reacts with oxygen radicals, forming a highly reactive metabolite, peroxynitrite, which interacts with proteins and DNA, causing nitration of proteins (nitroative stress), DNA damage, further activation of NFκB, caspase-3, and vascular cell apoptosis [62,63].

The deleterious effects of AGEs on the vasculature can also be classified as either receptor-dependent or receptor-independent.

5.1. Receptor-independent effects of AGEs

Glycation of proteins and lipoproteins can alter their normal function by several mechanisms: change in molecular conformation, alteration in enzyme activity, changes in clearance and interference with receptor recognition [64]. It has been demonstrated that glycation of LDL particles on the apolipoprotein B (ApoB) and phospholipid components [65,66] contributes to the exacerbation of atherosclerosis. Higher levels of glycated LDL (AGE-LDL) were found in patients with diabetes and suggested to belong to the features of dyslipidemia related to diabetes mellitus and renal insufficiency [66,67]. Glycation of ApoB results in impairment of LDL receptor-mediated uptake with subsequently reduced clearance of LDL [68]. In contrast, AGE-LDL uptake by macrophages is greater than that of native LDL by an LDL-receptor-independent mechanism involving a high capacity, low-affinity scavenger receptor [69]. Glycation enhances LDL uptake by human aortic intimal cells [70] and monocyte-derived macrophages [69], thereby stimulating the formation of foam cells and promoting atherosclerosis [64]. Alternatively, glycation of LDL promotes atherosclerosis via the increased susceptibility of AGE-LDL for oxidation, another critical pathway in the development of atherosclerosis [64,68,71]. A large body of evidence links ‘post-translational’ modifications such as glycation and oxidation of plasma lipoproteins to vascular mechanisms implicated in the pathogenesis of diabetic retinopathy. Under physiologic conditions, the inner and outer blood–retina barriers prevent the extravasation of plasma components [72,73]. Enhanced leakage is present even before and further increases with the manifestation of diabetic retinopathy in proportion with disease severity [72]. Several studies reported large amounts of modified LDL in the retina of persons with diabetes and its toxicity for retinal capillary pericytes [72], endothelial cells [74] and Müller cells [75]. Heavily-oxidized glycated LDL and HDL can impair retinal pigment epithelial cells by inducing a generation of reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, autophagy, apoptosis and decreasing glutathione peroxidase 1 (GPX-1) [73]. GPX-1 is considered the most important cytosolic and mitochondrial antioxidant enzyme in humans, detoxifying hydrogen peroxide and lipid peroxides [76]. On the other hand, glycation and oxidation of HDL decrease its antioxidant properties of suppressing the enhanced ROS generation induced by oxidized and glycated LDL, thereby further reducing the cellular antioxidant capacity. ER stress induces protein misfolding and triggers the “unfolded protein response” [77], which is implicated in the death of retinal neurons and vascular cells in patients with diabetes [78]. The ER is sensitive to oxidative stress induced by oxidized LDL as well as heavily oxidized glycated LDL [73,79] and prolonged ER stress induces ultimately apoptosis via activation of the pro-apoptotic transcription factor C/EBP-homologous protein (CHOP). Indeed, heavily oxidized glycated LDL markedly enhances CHOP expression in retinal pigment epithelial cells [73]. Autophagy plays a major role in intracellular homeostasis by removing damaged organelles, cell membranes and proteins, as well as preserving nutrients [80]. However, prolonged ER stress may switch autophagy from a protective role to one promoting cell death. Autophagy was
shown to mediate the deleterious effects of heavily oxidized glycated LDL on Muller cells, retinal pigment epithelial cells and retinal pericytes [73,75,79]. Taken together, oxidation and glycation of LDL seems to potentiate its pathogenic role, while oxidized glycated HDL partly loses its protective role.

Last but not the least, glycation of matrix proteins – collagen VI, laminin and vitronectin – increases the turnover of heparane sulfate, which in turn stimulates the compensatory overproduction of other matrix components [81]. Modification of matrix proteins by glycation also affects the interaction of transmembrane integrin receptors with some matrix ligands, altering stability. For example, the modification of the cell-binding domains of type VI collagen decreases endothelial cell adhesion [64,81].

5.2. Receptor-dependent effects of AGEs

AGE receptors are present on the surface of different cell types: macrophages, adipocytes, endothelial cells, and vascular smooth muscle cells (VSMC) [7,59,82–85]. Several types of receptors including scavenger receptors (macrophage scavenger receptor-AI, macrophage scavenger receptor-AII, CD68, and CD36), RAGE, AGE-R1, AGE-R2, and AGE-R3 have been described [86,87]. Although scavenger receptors are responsible for the removal of AGEs, RAGE likely mediates most biological effects of AGEs [88]. RAGE is a member of the immunoglobulin multiligand receptor family, which is involved in intracellular signal transduction. Its activation promotes inflammatory responses, apoptosis, prothrombotic activity, expression of adhesion molecules, and oxidative stress [59,60,89].

\[ \text{Figure 2: Receptor-dependent pathways activated by AGEs. A substrate excess of AGEs leads to a RAGE overexpression and exacerbation of RAGE-dependent pathways (a), while a low AGEs substrate availability activates mainly AGER1-triggered signals, AGEs endocytosis and degradation (b). Adapted from Poulsen et al., Food and Chemical Toxicology, 2013.} \]
The interaction between AGEs and RAGE activates several intracellular signaling cascades, for example the family of mitogen-activated protein (MAP) kinase, members of the JAK-STAT signaling family, CDC42, RAC1 and other members of the Ras family, SRC1, members of the SMAD signaling family and phosphoinositide 3-kinase.[59,89–91]. Moreover, RAGE signaling activates the central transcription factors [89], nuclear factor (NF)-ƙ [59], cAMP-response-element-binding protein (CREB)-1 [92], early growth response (EGR)-1 [93] and activator protein (AP)-1 [94] (Figure 1). Recent articles provide insights into the factors influencing AGEs–RAGE binding, as well as into RAGE structure, highlighting the importance of receptor oligomerization for the formation of active signaling complexes [95–98]. However, the precise mode of oligomerization remains unclear in the context of various ligands. Several of the RAGE ligands share structural features such as alpha and beta sheets, suggesting that RAGE recognizes three-dimensional structures [88,99]. The detailed mechanisms and consequences of RAGE activation are discussed in detail in recent comprehensive reviews [6,59,89,100,101]. Here we focus on its relevance for cardiovascular disease. Briefly, RAGE is up-regulated in atheromatous lesions [102] and in diabetes [103], suggesting that AGES-mediated pathomechanisms are exacerbated in these diseases [104].

Figure 3: The cycle of endogenous and exogenous AGES.

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Which AGES or which of their precursors bind to RAGE is still a matter of debate. N(ɛ)-carboxy-methyl-lysine (CML), N(ɛ)-carboxy-ethyl-lysine (CEL) and methylglyoxal have been suggested to bind to RAGE [98,105–107], but others have questioned whether AGES containing CML modifications [108] or early glycation adducts (fructosyllysine peptides) [98] can activate RAGE. Taking into consideration the multitude of existing AGES, further studies are warranted to clarify which AGES or AGES classes bind to RAGE and which do not.

Of note, RAGE does not exclusively bind AGES, but also non-AGES substrates like some members of the S100/calgranulin family of pro-inflammatory and migration-stimulating molecules [89,109]. Recently, Poulsen et al. [110] discussed the relevance of the in vivo interaction between AGES and RAGE by highlighting that the interaction between AGES (mainly CML) and RAGE does not uniformly induce an inflammatory response [108,111–113]. This could be due to the existence of splice variants of RAGE on certain cells [112], the fact that not all AGES interact with RAGE [108] or the presence of endotoxin in some preparations of AGES [111,113]. There are two further aspects that might mitigate the importance of AGES–RAGE interaction in vivo. The AGES–RAGE interaction seems to depend on the molecular size of the AGES compounds. In addition, the affinity of AGES for RAGE might be lower in vivo as compared to in vitro studies [110]. Indeed, comparing the affinity of low-molecular-weight (free or < 30 kDa) and high-molecular-weight CML binding to RAGE revealed that only the high-molecular weight CML interacts with RAGE [98,106,114].

Since only low-molecular weight AGES are absorbed from the gut, one can hypothesize that diets enriched in AGES will give rise only to low-molecular weight AGES in the circulation with scarce AGES–RAGE interaction. In contrast, Birlouez-Aragon et al. [115] showed that AGES-rich diets increase circulating high-molecular-weight AGES, suggesting that dietary AGES can induce the endogenous formation of larger AGES capable of RAGE activation. In line, Vlassara et al. [30] provide
evidence that diets with a high AGEs content given for several weeks induce an inflammatory response, typical for the AGEs–RAGE interaction. Nevertheless, RAGE seems to have more complex roles than we believe at the moment, since mice lacking RAGE show increased bone mass and bone mineral density and decreased bone resorption in vivo, suggesting a role of RAGE in osteoclast maturation and function [116]. Moreover, RAGE-dependent responses might aim at rapidly responding to vascular injuries and facilitate repair under physiological conditions, but set in motion a vicious circle of injury as soon as exacerbated AGEs concentrations occur [100].

6. EFFECTS OF AGES IN CELLS CONTRIBUTING TO ABNORMAL VASCULAR FUNCTION

Studies in animal models and in vitro have shown that AGEs impact the function of different cell types implicated in atherosclerosis: endothelial cells [54,63,117], platelets [118], monocytes/macrophages [119] or vascular smooth muscle cells [120], thus explaining their association with vascular disease [121].

6.1. Effects of AGES on endothelial cells, pericytes and angiogenesis

AGES’ interaction with RAGE-expressing endothelial cells results in reduced endothelial barrier function with increased permeability and subendothelial lipid entry. The AGEs–RAGE interaction triggers the expression of adhesion molecules, e.g. VCAM-1 [122], and promotes transendothelial migration of monocytes, thereby contributing to the formation of foam cells [122–124].

Xu et al. [53] demonstrated that the incubation of human umbilical vein endothelial cells (HUVEC) with AGEs-modified albumin, but not with a high-glucose medium, exerted a concentration- and time-dependent suppression of the endothelial isoform of the NO-synthase (NOS). NOS suppression was biphasic with a rapid component due to suppression of its serine phosphorylation and a slower component involving decreased NOS expression.

Many endothelial cell processes are dependent upon intracellular Ca\(^{2+}\) and Ca\(^{2+}\)-signaling, among them the synthesis of NO from arginine by the enzyme NOS. A study by Naser et al. [125] showed in bovine aortic endothelial cells that incubation with AGEs for 5 minutes causes an acute intracellular store depletion of Ca\(^{2+}\), such that the Ca\(^{2+}\) signal stimulated by the subsequent application of other agents acting upon that store was reduced. The same authors suggested as one of the mechanisms the generation of ROS from NAD(P)H oxidase.

Impaired angiogenesis of the peripheral vasculature contributes to delayed wound healing, exacerbated peripheral limb ischemia, and exacerbates cardiac morbidity via reduction of collateral vessel development [126]. Peppa et al. showed in genetically diabetic mice that dietary AGEs from a high-AGES (H-AGES) diet exert adverse effects on wound healing compared to a low-AGES (L-AGES) diet [127]. Wounds of L-AGES-treated mice exhibited lower skin AGEs deposits, increased epithelialization, angiogenesis, inflammation, granulation tissue deposition, and enhanced collagen organization compared with H-AGES mice. Interestingly, the modality of wound healing seemed to be influenced by food AGEs with re-epithelialization being the dominant mode of wound closure in H-AGES-fed mice compared with wound contraction that prevailed in L-AGES-fed mice.

The mechanisms of impaired angiogenesis induced by AGEs were partly explained by Liu et al. [107], who demonstrated in vitro using endothelial cells and mouse aortas that methylglyoxal (the highly reactive AGEs precursor) reduces endothelial angiogenesis through RAGE-mediated, peroxynitrite-dependent and autophagy-induced vascular endothelial growth factor receptor 2 (VEGFR2) degradation. VEGFR2 represents the principal receptor for vascular endothelial growth factor signaling, which leads to vasodilatation, endothelial cell migration and proliferation [107,128,129].

Apoptosis of retinal capillary pericytes and endothelial cells is one of the pathomechanisms contributing to the progression of diabetic retinopathy [130]. Nagaraj et al. [131] have shown in retinas from alloxan-induced diabetic dogs, intense AGES-specific modifications of the basement membrane proteins of retinal capillaries. Considering at the mechanisms using in vitro cultured bovine retinal capillary pericytes and HUVECs incubated with MG, the authors found an apoptosis of retinal pericytes induced by MG that was increased by 55% compared to pericytes incubated on a fibronectin media without MG. The authors proposed that the enhanced apoptosis occurred through the p38MAPK-mediated oxidative pathway and was accompanied by marked actin degradation and a dramatic decrease in αB-crystallin, a small heat shock protein present mainly in the eye lens. While this study suggested that pericytes’ apoptosis occurs because of the surrounding basement membrane protein modification by AGEs, other studies suggested that pericytes apoptosis is due to the effects of circulating AGEs, resulting in VEGF overproduction [132] and binding to surface AGEs-receptors [133].

6.2. Effects of AGES on platelets

Platelets activated by different agonists (ADP, collagen and thrombin) express P-selectin (CD62), an adhesion molecule on the surfaces of activated endothelial cells and activated platelets, and adhere to endothelial cells and blood leukocytes as a key event in the sequence of thrombus formation, inflammatory reaction [134] and in the development of atherosclerotic lesions and atherothrombosis [135].

AGEs strongly activate platelets obtained from both healthy humans and patients with type 2 diabetes [118]. AGEs stimulate the expression of CD62 up to 7.1-fold and CD63, a cell surface glycoprotein forming complexes with integrins and a potential blood platelet activation marker, up to 2.2-fold at the platelet surface membrane in a concentration- and time-dependent manner. Given the postprandial increase in circulating AGEs [136,137], this might explain the postprandial frequency of acute ischemic events. In this study, platelet activation by AGEs was accompanied by an increase in platelet RAGE expression without proof of an AGEs interaction.

Platelets contain specific storage granules, which fuse with the plasma membrane during degranulation. This mechanism can possibly lead to the translocation of RAGE to the platelet surface. However, additional studies are required to examine the AGEs interaction with RAGE or other receptors for AGEs on a platelet surface. Of note, Zhu et al. [138] demonstrated an interaction between AGEs and CD36 on platelet surfaces resulting in a prothrombotic phenotype.

6.3. Effects of AGES on inflammatory cells

Binding of AGEs to RAGE on the surface of monocytes induces the production of mediators: interleukin-1, tumor necrosis factor-α, platelet-derived growth factor and insulin-like growth factor-1, resulting in chemotaxis and inflammatory response [119,139–141]. Kirstein et al. [119] showed that (i) in vitro- and in vivo-formed AGEs are chemotactic for human blood monocytes, (ii) sub-endothelial AGEs can selectively induce monocyte migration across an intact endothelial cell monolayer, and (iii) subsequent monocyte interaction with AGEs-containing matrix results in the expression of platelet-derived growth factor. These authors highlighted that their findings support the hypothesis that AGEs can act as signals for the normal turnover of
senescent tissue protein by means of the AGES-specific receptor system. Also, AGES increase the expression of blood-borne tissue factor (TF) by cultured or fresh isolated monocytes as shown in different studies [142,143]. Bierhaus and colleagues found that the induction of TF expression by cultured endothelial cells with AGES is mediated via the AGES–RAGE interaction [144]. High expression of TF on monocytes and vascular endothelium appears to regulate the inflammatory response [145,146]. Adherence of activated platelets to leukocytes is a key event in the sequence of thrombus formation, and platelet recruitment by activated leukocytes plays an important role in modulating an inflammatory reaction [134]. Hu and co-workers have shown that diabetic individuals show elevated circulating platelet-leukocyte aggregates in blood [147]. Gawlowski et al. demonstrated that glycated albumin and MG induce Mac-1 expression on neutrophils, which results in enhanced formation of platelet–leukocyte aggregates [143].

Zhang and coworkers [148] found in pigs with streptozotocin-induced diabetes that diabetes increased NAD(P)H oxidase activity and oxidative stress, producing inflammatory responses in porcine coronary media and adventitia. They examined the underlying mechanisms in isolated coronary fibroblasts and demonstrated that AGES, rather than glucose itself, upregulate the expression of interleukin-6, VCAM-1, and monocyte chemotactic protein-1 mRNAs. These changes were paralleled by increased interleukin-6 secretion and augmented leukocyte adhesion to AGE-stimulated coronary cells. Furthermore, AGES increased the expression of phosphorylated forms of mitogen-activated protein kinases in coronary cells (ERK1/2 and JNK) and resulted in redox-sensitive expression of inflammatory genes. These effects were inhibited by several inhibitors of oxidative pathways [NAD(P)H oxidase inhibitors, N-acetylcysteine, and pyrrolidine dithiocarbamate].

Wang et al. [149] studied cultured macrophages, A7r5 aortic smooth muscle cells, in vitro as well as an animal model of arteriosclerosis and diabetes. Streptozotocin (STZ)-diabetic male apoE(–/–) mice were fed a semi-synthetic high-fat diet (HFD) plus daily injections of CML (10 mg/kg/day). The STZ-CML-HFD condition resulted in early atherosclerotic plaques at 2 months and typical advanced plaques at 4 months. Deposition of CML and expression of RAGE in the aortic wall were mainly restricted to the atherosclerotic plaques. Both animal and cell studies consistently demonstrated that the CML/RAGE axis may first initiate apoptosis of macrophages in atherosclerotic lesions and then induce bone morphogenetic protein 2 (BMP-2)-core-binding factor α1 (cbfα1) and alkaline phosphatase (ALP)-calcification dependent cascade in a high lipid, apoptosis-coexisting environment. From these data, the authors suggested that the CML–RAGE interaction plays an important role in atherosclerotic calcification in diabetes, through the induction of macrophages’ apoptosis, followed by the osteogenetic differentiation of aortic smooth muscle cells.

6.4. Effects of AGEs on vascular smooth muscle cells (VSMC)

There is also evidence that the activation of RAGE on VSMC promotes cellular proliferation by mechanisms likely mediated by cytokine or growth factors [64,150]. Neointima formation in response to arterial injury involves VSMC proliferation and migration, potentially leading to arterial stenosis, thrombosis and ischemia in atherosclerosis, hypertension and arterial revascularization. RAGE activation by glycated albumin induced in cultured VSMC increased proliferation and migration by suppressing adenosine monophosphate kinase (AMPK) activation [120]. Some further mechanisms linking RAGE activation to VSMC proliferation might be represented by extracellular signal-regulated kinase (ERK) activation [151–153], autophagy induction [152], the activation of signal transducer and activator of transcription (STAT)3/oncoprotein provirus integration site for Moloney murine leukemia virus (Pim1)/nuclear factor of activated T-cells (NFAT) axis [154], and activation of the serine/threonine kinase Akt1 [155]. Furthermore, activation of RAGE induces osteogenic differentiation of VSMC and thereby promotes vascular calcification through Notch/Msx2 induction [156].

Finally, accumulation of the membrane attack complex of complement (MAC) in blood vessels promotes proliferation of fibroblasts and VSMC [157]. While the regulatory membrane protein CD59 normally restricts MAC accumulation, its glycation results in MAC inactivation, as well as in the atherosclerosis-promoting proliferation of fibroblasts and VSMC [158].

7. VASCULAR EFFECTS OF AGES IN ANIMAL MODELS

The detection of AGES and overexpressed receptors for AGES in atheromatous plaques and lesions, as well as within lipid accumulations in VSMC and macrophages from diabetic patients, has narrowed the path between AGES and atherosclerosis [102,159–161]. In genetically hypercholesterolemic apolipoprotein E-deficient (apoE(−/−)) streptozotocin-induced diabetic mice, a diet with a low AGES content compared to an isocaloric standard diet with a high AGES content resulted in > 50% smaller lesions at the aortic root after 2 months. Only serum AGES differed by about 53%, while no differences were noted in plasma glucose, triglycerides, or cholesterol levels between both groups [162].

Insulin resistance and reduced levels of HDL are prominent risk factors for cardiovascular disease and dietary AGES were shown to increase circulating insulin levels and decrease circulating HDL levels in the db/db mouse model [163].

In another mouse model of genetic hypercholesterolemia, Lin et al. [164] showed that lowering of dietary AGES reduces neointimal formation after arterial injury and suggested that dietary restriction of AGES may have a potential role in the prevention of restenosis after angioplasty. Vlassara et al. [165] administered AGE-modified albumin to nondiabetic rats and rabbits, alone or in combination with the AGE-crosslink inhibitor aminoguanidine. AGES treatment for 2–4 weeks led to a sixfold higher content of AGES content in aortic tissue samples compared to untreated controls, which was accompanied by increased vascular permeability and markedly impaired vasodilatory responses to acetylcholine and nitroglycerin. These effects were significantly reduced by treatment with aminoguanidine. Moreover, mononuclear cell migratory activity was enhanced in subendothelial and periarteriolar regions of various tissues from AGES-treated rats when compared to tissues from animals treated with aminoguanidine. The authors concluded that AGES can induce complex vascular alterations resembling those seen in diabetes or aging, independent of metabolic or genetic factors, and that these changes can be reversed by the AGE-crosslink inhibitor aminoguanidine.

In rats, aminoguanidine administration was also effective in preventing diabetes-induced formation of fluorescent advanced nonenzymatic glycosylation products and cross-linking of arterial wall connective tissue protein in vivo [166]. Furthermore, aminoguanidine treatment for 18 months prevented increases in the AGES content in aged cardiac, aortic, and renal tissues, markedly inhibited age-related albuminuria and proteinuria as well as age-related cardiac hypertrophy and preserved endothelium-dependent and endothelium-independent vascular function in non-diabetic female Sprague-Dawley and Fischer 344 rats [167]. Soluble RAGE (sRAGE, composed of the extracellular ligand-binding domain of RAGE), binds AGES and blocks their interaction with RAGE,
thus partly reducing the detrimental effects of AGEs (Figure 2). A series of studies demonstrated that treatment with sRAGE (Figure 2) resulted in dose-dependent reduction of the atherosclerotic lesion area and complexity, together with a decrease in AGEs, vascular inflammation and oxidative stress in apoE-null mice, while plasma glucose, cholesterol and triglyceride concentrations remained similar to vehicle treated mice [168,169]. In another study, administration of sRAGE stabilized already existing atherosclerotic lesions [170]. Finally, Wendt et al. [171] bred apo E−/− mice into the db/db background and treated them with sRAGE. These animals had significantly reduced atherosclerotic lesion area independent of the prevailing glycaemia and lipidemia, thereby highlighting an important role of AGEs–RAGE interaction in mediating proatherogenic mechanisms.

These studies showed that higher circulating concentrations of AGEs, particularly food-derived AGEs, can induce cross-linking of arterial wall connective tissue [166], aortic atherosclerotic lesions [162], neointimal formation after arterial injury [164], increase vascular permeability and markedly impair vascular vasodilatory response [165]. On the other hand, inhibitors of AGEs such as aminoguanidine or soluble RAGE can counteract diabetes- or age-induced effects of AGEs on the vasculature, suggesting that dietary or therapeutic interventions can be effective — at least in animal models — in preventing some key proatherosclerotic mechanisms.

8. VASCULAR EFFECTS OF AGES IN CLINICAL STUDIES

Studies in animal and cellular models have provided evidence for the implication of AGEs in vascular disease and explained some of the underlying mechanisms. In humans, several clinical studies have linked AGEs to cardiovascular disease, particularly in patient with diabetes mellitus. Kiuchi and coworkers [172] reported higher serum AGE concentrations in type 2 diabetes patients with coronary artery disease (CAD) than in patients without obstructive CAD and higher than in normoglycemic patients with and without obstructive CAD. Similar results were reported by Kilhovd et al. [173] and Aso et al. [174]. Moreover, serum AGEs were found to be associated with the degree of CAD in patients with type 2 diabetes and obstructive CAD. Of note, elevation of serum AGEs in patients undergoing percutaneous coronary intervention was identified as an independent risk factor for restenosis in diabetes mellitus [175]. Prospective studies also support the association of AGEs with cardiovascular disease (CVD). In type 1 diabetes, circulating AGEs were associated in a 12-years follow-up study with incident fatal and nonfatal CVD as well as all-cause mortality [3]. In female, but not in male type 2 diabetes patients, elevated serum AGEs predicted mortality due to CAD during a follow-up of more than 18 years [176]. These authors reported a similar gender-specific pattern, in that serum levels of AGEs predicted both total and CAD-induced mortality in normoglycemic women [177]. Studying patients with chronic heart failure (CHF) with only 9% also having diabetes, Hartog et al. [178] demonstrated that CML predicted the composite outcome (death, heart transplantation, ischemic cardiovascular events and hospitalization due to heart failure) even after adjustment for age, gender, etiology of heart failure and several other known predictors of CHF outcome.

The above-mentioned studies linked high AGEs concentrations to cardiac disease and its outcome. But these studies could not prove a causal relationship, so that the question remained unsolved whether AGEs are innocent bystanders or contribute to the development of cardiovascular disease. We demonstrated that changing the cooking method alone (and thereby changing the amount of ingested AGEs), has a relevant transient impact on postprandial microvascular and macrovascular endothelial function in persons with type 2 diabetes mellitus [137]. On the other hand, we recently reported that acute oral administration of AGEs alone transiently impairs endothelial function as an early marker of atherosclerosis [179–181], supporting the concept that nutritional AGEs can have a direct detrimental effect on vasculature in humans [182].

Noninvasive measurements have shown that skin autofluorescence (SAF) is increased in people with increased cardiovascular risk such as people with carotid artery stenosis and peripheral artery disease, independent of the presence of diabetes [183]. An association of SAF with the one-year incidence of major adverse cardiac events has also been reported [184]. SAF provides also additional information on the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in people with type 2 diabetes mellitus [185].

9. POTENTIAL THERAPEUTIC OPPORTUNITIES TARGETING AGE-RELATED PROCESSES

In line with our data [137], several studies found that restriction of dietary AGEs was accompanied by a fall in circulating AGEs and a decrease in markers of oxidative stress, inflammation, endothelial dysfunction and insulin resistance in patients with diabetes mellitus [30,186], renal failure [31] as well as in healthy persons [115]. Since changing the cooking method (e.g. reducing temperature, increasing humidity, decreasing the pH) is highly effective in influencing the amount of AGEs contained in food [29], this might represent a simple, meaningful and cost-effective intervention against the AGEs burden.

Thiamine and its prodrug benfotiamine activate the enzyme transketolase, thus activating the pentose-5-phosphate glycolytic path and blocking other pathways involved in the development of diabetes complications, among them the production of AGEs [187]. Clinical and experimental studies have strengthened these findings [136,188,189] but endpoint studies are still awaited.

Besides thiamine, sRAGE and aminoguanidine, many other substances were attributed roles against AGEs: drugs specifically developed as AGEs inhibitors (pyridoxamine, hydrazono-oxo-thiazolidine, 2,3-diaminophenazine), AGEs breakers (N-phenacylthiazolium, pyridoxamine), anti-inflammatory drugs with anti-glycation properties (e.g. tenilsetam, diclofenac, aspirin), renoprotective drugs with AGEs inhibition activity (angiotensin-converting enzyme-inhibitors, angiotensin-II receptor blockers, hydralazine), metabolically active drugs and vitamins with anti-AGEs properties (pyridoxamine, metformin, aldose reductase inhibitors), antioxidants and free radical trapping agents (carnosine, flavonoid-rich herbal extracts, curcumin), and AGEs inhibitors with chelating properties (carnosine, pyridoxamine, temocaprilat, tenilsetam, cefotazidin, pioglitazone, acarbose, etc.) [190,191]. However, many of these substances need validation by clinical studies.

10. CONCLUSIONS

Increases in AGEs can result from increased endogenous production under conditions of hyperglycemia or from exogenous dietary sources. AGEs exert their deleterious effects on the vasculature by both receptor-dependent and receptor-independent mechanisms. The receptor for AGEs, RAGE, seems to mediate most of the biological effects of AGEs by generating ROS and stimulating inflammatory pathways. In diabetic
different cells implicated in atherosclerosis, thereby reducing the activity of diabetes-related hyperalgesia[192]. AGEs also modify the function of NOS and intracellular Ca2+-dependent signaling by endothelial cells, activating platelets, increasing chemotaxis and inflammation mediated by monocytes/macrophages or promoting cellular proliferation and osteogenic differentiation of VSMC.

The results of the DCCT-EDIC study have strengthened the role of the so-called “glycemic memory”, postulating that metabolic control over several years predicts the development of diabetic complications [193]. The fact that glycated proteins form under hyperglycemic conditions and chronically accumulate within organs that are prone to diabetes-related complications suggests AGEs as ideal candidates mediating the “glycemic memory” [150,194,195]. On the one hand, inhibitors of AGEs (aminoguanidine, thiamine, benfotiamine, pyridoxamine or soluble RAGE) can counteract diabetes- or age-induced effects of AGEs on vasculature in preclinical studies. On the other hand, several studies have shown that dietary restriction of AGEs is feasible in patients with diabetes or renal failure as well as in healthy persons and results in marked decrease in circulating AGEs and markers of oxidative stress, inflammation, endothelial dysfunction and insulin resistance. Taken together, there is compelling evidence for a detrimental role of AGEs in driving vascular disease. Nevertheless, better understanding of the underlying mechanisms will be paramount for the development of treatment strategies targeting AGEs and thereby aiming at improving morbidity and mortality in persons with diabetes mellitus.

CONFLICT OF INTEREST

Authors have no conflict of interest to disclose related to the topic of this article.

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