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Review

Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes

Giuseppina Basta[a], Ann Marie Schmidt[b], Raffaele De Caterina[a,c,*]

[a] *CNR Institute of Clinical Physiology, Pisa, Italy*
[b] *Columbia University, New York, NY, USA*
[c] *Institute of Cardiology and Center for Excellence on Aging, "G. d'Annunzio" University, c/o Ospedale S. Camillo de Lellis, Via Forlanini, 50, I-66100 Chieti, Italy*

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Abstract

The formation of advanced glycation end products (AGEs) is an important biochemical abnormality that accompanies diabetes mellitus and, likely, inflammation in general. Here we summarize and discuss recent studies indicating that the effects of AGEs on vessel wall homeostasis may account for the rapidly progressive atherosclerosis associated with diabetes mellitus. Driven by hyperglycemia and oxidant stress, AGEs form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGEs may "trap" plasma proteins, quench nitric oxide (NO) activity and interact with specific receptors to modulate a large number of cellular properties. On plasma low density lipoproteins (LDL), AGEs initiate oxidative reactions that promote the formation of oxidized LDL. Interaction of AGEs with endothelial cells as well as with other cells accumulating within the atherosclerotic plaque, such as mononuclear phagocytes and smooth muscle cells (SMCs), provides a mechanism to augment vascular dysfunction. Specifically, the interaction of AGEs with vessel wall components increases vascular permeability, the expression of procoagulant activity and the generation of reactive oxygen species (ROS), resulting in increased endothelial expression of endothelial leukocyte adhesion molecules. AGEs potently modulate initiating steps in atherogenesis involving blood-vessel wall interactions, triggering an inflammatory-proliferative process and, furthermore, critically contribute to propagation of inflammation and vascular perturbation in established disease. Thus, a better understanding of the biochemical mechanisms by which AGEs contribute to such processes in the vessel wall could be relevant to devise preventive and therapeutic strategies for diabetic atherosclerosis.

Keywords: Advanced glycation end products; Atherosclerosis; Inflammation; Diabetes; Endothelium

1. Introduction

Both type-1 and type-2 diabetes are powerful and independent risk factors for coronary artery disease, stroke, and peripheral arterial disease [1,2]. Accelerated atherosclerosis, as well as microvascular disease, are the major vascular complications of diabetes, constituting the main cause of morbidity and mortality in this common metabolic disorder.

The primary causal factor leading to the pathophysiologic alterations in the diabetic vasculature is the chronic exposure to high levels of blood glucose [3]. A causal relationship between chronic hyperglycemia and diabetic microvascular disease, long inferred from a variety of animal and clinical studies [4], has been definitely established by data from the Diabetes Control and Complications Trial, comprising two multicenter, randomized, prospective controlled clinical studies [3,5]. A relationship between chronic hyperglycemia and diabetic macrovascular disease in non-insulin-dependent diabetes mellitus patients is also supported by a number of other reports [6,7].

Although the effects of glucose in adversely modulating cellular properties occurs by a variety of mechanisms [8,9], the most important pathway involved in the pathogenesis of the accelerated atherosclerosis in diabetes is most likely the increase in nonenzymatic glycation of proteins and lipids, with the irreversible formation and deposition of reactive advanced glycation end products (AGEs). This

* Corresponding author. Tel.: +39-0871-41512; fax: +39-0871-402817.
E-mail address: rdecater@unich.it (R. De Caterina).

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review specifically focuses on proposed mechanisms by which AGEs accumulate in the extracellular space and within cells of the vessel wall and thus contribute to the accelerated atherosclerosis in diabetes.

AGEs may promote atherogenesis by oxidizing low density lipoproteins (LDL) and causing changes in the intimal collagen. A major contribution of AGEs to atherogenesis however recently emerges from important studies that have led to the isolation of a receptor for AGEs on cell surface, termed ''RAGE'', which functions as a signal transduction receptor, also binding non-AGE-related pro-inflammatory molecules such as S100/calgranulins and amphoterins. The overlapping accumulation and expression of RAGE and its ligands at sites of tissue lesions sustains RAGE-mediated cellular activation and the induction of multiple signaling pathways. The importance of RAGE–ligand interaction is underscored by the suppression of early accelerated atherosclerosis and established atherosclerosis in a glycemia- and lipid-independent manner in diabetic apolipoprotein-E (apo-E) null mice after treatment with the soluble extracellular domain of the receptor for AGEs. All such notions will be here reviewed in detail.

2. Biochemical mechanisms leading to production of AGEs

Nonenzymatic glycation occurs through the covalent binding of aldehyde or ketone groups of reducing sugars to free amino groups of proteins, forming a labile Schiff's base (Fig. 1).

The initial Schiff's base undergoes rearrangements to a much more stable ketoamine, called Amadori's product (Fig. 1). The reactive free carbonyl group of these Amadori's products is responsible for some of the biological consequences of glycation. In addition, Amadori's products can be degraded into a variety of other highly reactive carbonyl compounds such as 3-deoxy-glucosone, which can react again with free amino groups to form intermediate glycation products. Recently, it has been proposed that the intermediates contributing to AGE formation include dicarbonyl intermediates such as 3-deoxy-glucosone, glyoxal and methyl-glyoxal [10] (Fig. 1). Glyoxal and methyl-glyoxal can be also formed by glucose auto-oxidation and by products from glycolipids [11,12]. These initial and intermediate glycation products slowly undergo

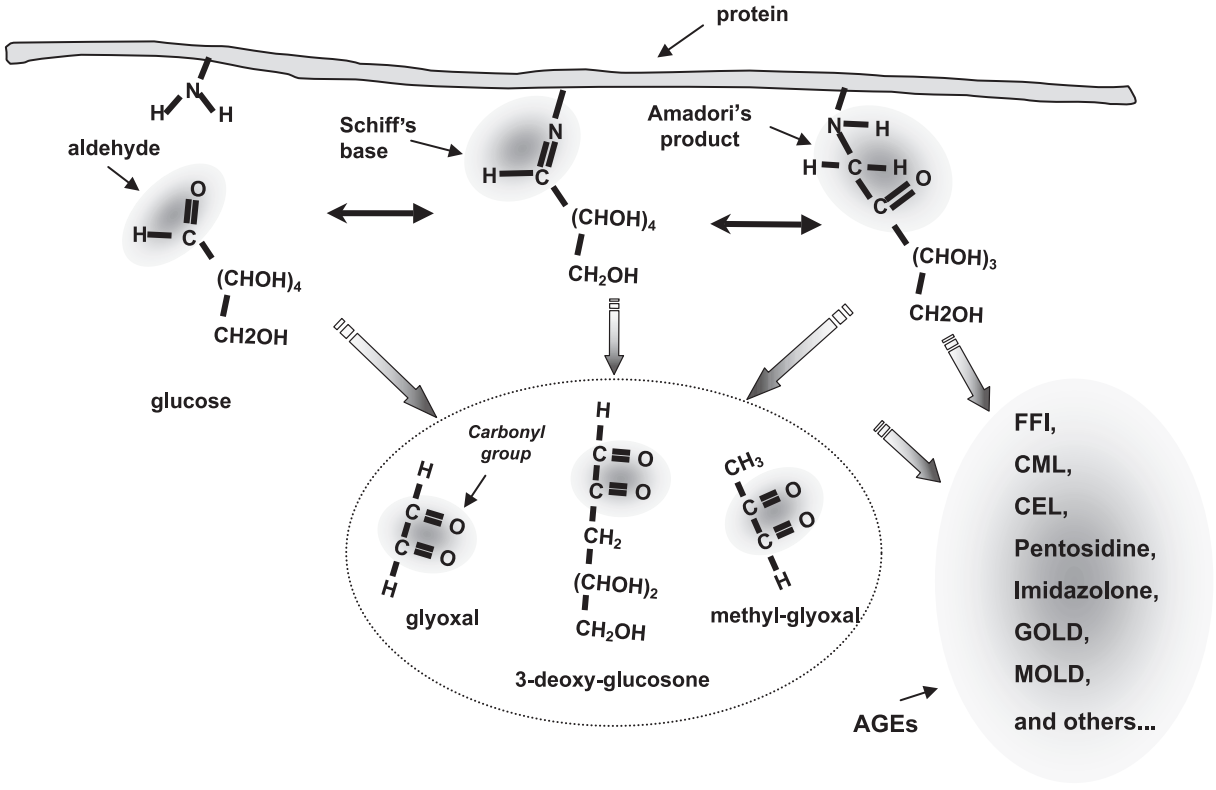

Fig. 1. Possible pathways in the formation of advanced glycation end products (AGEs). The initial interaction between the highly reactive aldehyde group of glucose with any free amino group on proteins creates a Schiff's base, which spontaneously rearranges itself into an Amadori's product. Subsequent, slower changes (not shown) are progressively less reversible, and ultimately lead to the formation of AGEs. In addition, a variety of highly reactive carbonyl intermediates such as 3-deoxy-glucosone, glyoxal and methyl-glyoxal can be formed by glucose or Schiff's base or Amadori's product auto-oxidation, which can react again with free amino groups to form AGE products such as imidazolone, *N*-ε-carboxy-methyl-lysine (CML), *N*-ε-carboxy-ethyl-lysine (CEL), glyoxal-lysine dimer (GOLD) and methyl-glyoxal-lysine dimer (MOLD).

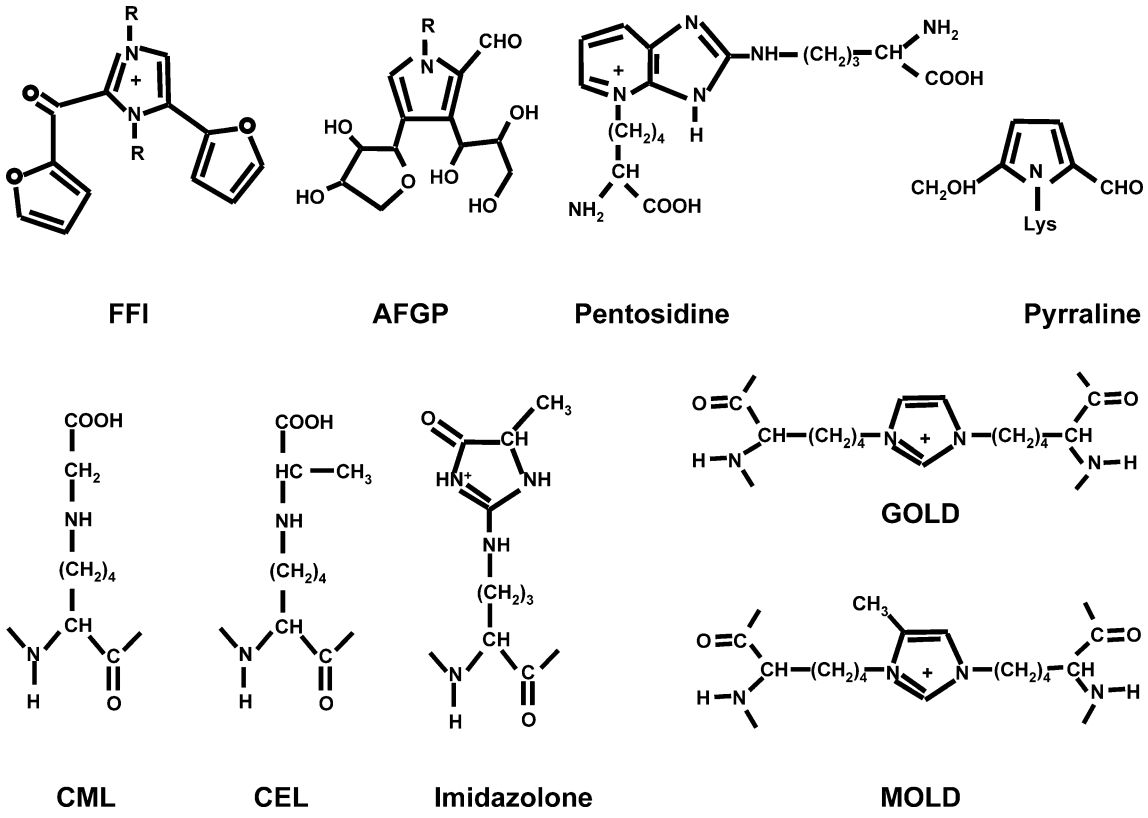

Fig. 2. Chemical structures of some advanced glycation end products (AGEs). FFI: 2-(2-furoyl)-4(5)-furanyl-1*H*-imidazole; AFGP: 1-alkyl-2-formyl-3,4-diglycosyl pyrrole; Pentosidine: pyrraline; CML: *N*-ε-carboxy-methyl-lysine; CEL: *N*-ε-carboxy-ethyl-lysine; Imidazolone; GOLD: glyoxal-lysine dimer; MOLD: methyl-glyoxal-lysine dimer.

a complex series of further chemical rearrangements, to yield irreversible AGE structures of yellow-brown color and fluorescence, with a propensity to generate reactive oxygen species (ROS) and interact with specific cell surface structures [13]. AGEs comprise a large number of chemical structures including: 2-(2-furoyl)-4(5)-furanyl-1*H*-imidazole (FFI), 1-alkyl-2-formyl-3,4-diglycosyl pyrroles (AFGPs), *N*-ε-carboxy-methyl-lysine (CML), pyrraline and pentosidine [14] (Fig. 2). Biochemical and immunohistochemical studies suggested that CML modifications of proteins are predominant AGEs that accumulate in vivo [15–17].

Recently, a significant new fraction of total AGEs, with relevant effects not only on protein structure and function, but also as mediators of biological responses, have been characterized in tissues. These compounds include: (1) the imidazolone adduct formed by reaction of 3-deoxy-glucosone with arginine residues in protein; (2) *N*-ε-carboxy-ethyl-lysine, an analogue of CML formed by the reaction of methyl-glyoxal with lysine; (3) glyoxal-lysine dimer (GOLD); and (4) methyl-glyoxal-lysine dimer (MOLD), which are imidazolium cross-links formed by the reaction of glyoxal or methyl-glyoxal with lysine residues in protein [18–21] (Fig. 2). In addition, the presence of white blood cell myeloperoxidase can enhance the formation of glycolaldehyde and 2-hydroxy-propanal from serine and threonine, respectively, even in the absence of sugars [22], suggesting a role for AGEs in inflammation [23,24].

3. How AGEs promote atherosclerosis: molecular mechanisms

In type-2 diabetic patients with coronary heart disease, elevated levels of AGEs and CML have been reported [25]. Immunohistochemical analyses of human atherosclerotic lesions using a monoclonal anti-AGE antibody have demonstrated diffuse extracellular as well as dense intracellular

Table 1
Non-receptor-mediated effects of AGEs on atherogenesis

Extracellular matrix
Collagen cross-linking and high resistance to collagenases [29]
Enhanced synthesis of extracellular matrix components [28]
Decreased polymer self-assembly of laminin and impairment of binding to type-IV collagen, and heparan sulfate proteoglycans [38]
Quenching of nitric oxide by collagen-linked AGEs [80]
Trapping of LDL and IgG in the subendothelium [35,36]
Lipoprotein modifications
Reduced AGE–LDL recognition by cellular LDL receptor [39]
Increased LDL susceptibility to oxidative modifications [39,41]

AGEs: advanced glycation end products; LDL: low density lipoproteins; IgG: immunoglobulins G.

Table 2
Receptor-mediated effects of AGEs on atherogenesis

Mononuclear phagocytes
Induction of PDGF, IGF-1, IL-1 β and TNF-α [67–69]
Chemotaxis by soluble AGEs [68,71]
Apoptaxis by immobilized AGEs [70,71]
Increased macrophage uptake of AGE–LDL [43]
Smooth muscle cells
Increased proliferative activity [73,74,88]
Increased production of fibronectin [73]
Endothelial cells
Increased permeability [75,76]
Increased intracellular oxidative stress [58,59,63]
Induction of endothelin-1 and increased vasoconstriction [82]
Reduction of thrombomodulin expression and induction of tissue factor expression [75,77]
Increased expression of adhesion molecules [23,61]

AGEs: advanced glycation end-products; PDGF: platelet-derived growth factor; IGF-1: insulin-like growth factor-1; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; LDL: low density lipoproteins.

AGE deposition in macrophages and vascular smooth muscle cells [26,27]. Tissue AGE concentration correlates with the severity of atherosclerotic lesions and with the accumulation of plasma proteins, lipoproteins and lipids in the vessel wall [25,28].

AGEs can be highly deleterious to the integrity and function of blood vessel walls in several ways. One possibility is the purely mechanical dysfunction caused by AGE cross bridges among vessel wall macromolecules [29]. A second form of damage promoting atherosclerosis is that AGE accumulation can cause circulating blood cells to adhere to the vessel wall. A third, nonmechanical source of damage is the perturbation of cellular function through binding to a variety of receptors that have been identified on various cell types, including macrophages, endothelial cells, smooth muscle cells, renal and neuronal cells [30–33].

AGE formation may thus accelerate the atherosclerotic process through two general mechanisms which can be classified as non-receptor-dependent (Table 1) and receptor-mediated (Table 2).

4. Non receptor-mediated effects of AGEs on atherogenesis

4.1. Effects of AGEs on extracellular matrix

AGE formation alters the functional properties of several important matrix molecules. Collagen in the blood vessel wall has a relatively long biological half-life, and with time undergoes significant nonenzymatic glycation, which may have a considerable bearing on atherosclerosis [34]. Soluble plasma proteins, such as LDL and immunoglobulins (Ig)G, are also entrapped and covalently cross-linked by AGEs on collagen [35,36].

AGE formation on type-IV collagen from the basement membrane inhibits lateral association of these molecules into a normal network-like structure [29,37]. Formation of AGEs on laminin decreases polymer self-assembly, as well as binding to type-IV collagen and heparan sulfate proteoglycans [38].

These AGE-induced abnormalities in the function of extracellular matrix alter structure and function of intact vessels.

4.2. Effects of AGEs on lipids

The glycation process occurs both on the apoprotein B (apoB) and on phospholipid components of LDL, leading to both functional alterations in LDL clearance and increased susceptibility to oxidative modifications [39]. In fact, diabetic LDL samples revealed significantly elevated levels of both apoB- and lipid-linked AGEs, which correlated with levels of oxidized LDL [40]. It has been proposed that intermediates such as glyoxales, glycolaldehydes, hydroxyaldehydes or other carbonyl group-containing compounds can be formed in the oxidation of both carbohydrates and polyunsaturated fatty acids [41]. These common intermediates, as mentioned above, can in turn react with free amino groups of proteins (such as LDL apoB) to form AGE products, including imidazolone, CML, CEL, GOLD, MOLD and others [19,20,42]. Further, the uptake of glycated LDL by human monocyte-derived macrophages occurs to a greater extent than for native LDL by a low-affinity nonspecific (“scavenger”) receptor with the resulting stimulation of “foam” cell formation, characteristic of the early atherosclerotic lesion [43].

5. Receptor-mediated effects of AGEs in atherogenesis

5.1. Cellular uptake of AGEs

Cell surface AGE-receptors mediate endocytosis and degradation of AGE-modified molecules, serving an important function in AGE catabolism and turnover. Search for mechanisms of AGE removal has led to the discovery of several cellular receptors binding these irreversibly modified macromolecules. Initially, it was observed that both in vivo-isolated and in vitro-synthesized AGEs are recognized by a macrophage AGE receptor that is distinct from previously described macrophage scavenger receptors [44]. Several studies have later led to the identification, cloning, and analysis of a receptor for AGEs (RAGE), which is until now the best characterized protein mediating cellular effects of AGEs [45–47]. RAGE, a multi-ligand member of the immunoglobulin superfamily, is increasingly viewed as an intracellular signal-transducing peptide rather than as a simple receptor involved in AGE endocytosis and turnover [48].

5.2. Structure of RAGE protein

RAGE is an approximately 45-kDa protein originally isolated from bovine lung endothelium on the basis of its

ability to bind AGE ligands [49]. Subsequent molecular cloning revealed that RAGE was a newly identified member of the immunoglobulin superfamily of cell-surface molecules [50]. The entire mature receptor consists of 403 amino acids in man, rat and mouse. The extracellular region of RAGE consists of one V-type (variable) immunoglobulin domain, followed by two C-type (constant) immunoglobulin domains, stabilized by internal disulfide bridges between cysteine residues. The V-type domain includes two putative N-linked glycation sites. In addition to the extracellular domain, RAGE displays a single putative transmembrane-spanning region and a short, highly charged cytosolic tail.

5.3. RAGE tissue expression, ligands and activation

RAGE has been found to be highly conserved across species and expressed in a wide variety of tissues [50–52]. Its presence in multiple tissues suggests a potential relevance of ligand–RAGE interactions for the modulation of vascular properties, as well as neural, renal and cardiac functions, prominently affected in diabetes and aging. Indeed, the expression of RAGE is upregulated at sites of diverse diseases, from atherosclerosis to Alzheimer's disease and Amyotrophic Lateral Sclerosis [45,53]. In this context, other ligands for the receptor have been identified and are linked to homeostatic as well as pro-inflammatory events. A first example for this is the binding to RAGE of amphoterin, a developmentally expressed neurite-outgrowth promoting protein that is, intriguingly, upregulated in tumors, where its interaction with RAGE facilitates tumor cell migration and invasion [30,54]. A second example is a polypeptide of the S100/calgranulin family of pro-inflammatory cytokines, S100A12 [55]. This latter, also termed *extracellular newly identified RAGE binding protein* (EN-RAGE), interacts with RAGE in a dose-dependent and saturable manner, resulting in the activation of cellular targets and competing with another member of the S100/calgranulin family, S100B, also capable of binding to RAGE [55]. Thus, RAGE is a receptor not only for AGEs, but also for S100/calgranulins, molecules found in any inflammatory lesion, including the blood vessel wall of diabetic individuals [56,57]. The overlapping presence of high levels of AGEs, S100/calgranulins, and RAGE, together with dyslipidemia, might conspire to cause the rapid atherosclerosis observed in diabetes. This property of RAGE of binding different seemingly diverse ligands deserves further research.

Another feature of RAGE is an unusual co-expression with its ligands in tissues. At sites of accumulated AGEs and S100/calgranulins in the vascular lesions, for example, there is increased expression of the receptor in cells of the vessel wall, including the endothelium, vascular smooth muscle cells and invading mononuclear phagocytes [57].

This overlapping distribution of the receptor and its ligands is thought to lead to prolonged cellular activation, resulting in further increased expression of the receptor. Contrary to other receptors, such as the LDL receptor, which are downregulated by increased levels of their ligand, the RAGE–ligand interaction would thus lead to a positive feedback activation, which further increases receptor expression. To date, the only way to substantially downregulate RAGE expression is to interrupt the cycle of ligand engagement of the receptor, by means of soluble RAGE or blocking antibodies.

5.4. Signal transduction pathways activated by RAGE–ligand interaction

The most important pathological consequences of RAGE–ligand interaction appear to be cellular activation, leading to the induction of oxidative stress and a broad spectrum of signaling mechanisms. Even if AGEs were nothing more than accidental ligands for RAGE, interaction of RAGE with other ligands such as amphoterin is likely to induce similar consequences.

In the vasculature, the principal pathological consequence of AGE interaction with endothelial surface RAGE is the induction of intracellular reactive oxygen species (ROS) [58], the generation of which seems to be linked, at least in part, to the activation of the NAD(P)H-oxidase system [59] (Fig. 3). These ROS would in turn activate the redox-sensitive transcription nuclear factor NF-κB, a pleiotropic regulator of many "response-to-injury" genes. This signal transduction cascade can be blocked by antibodies directed against either RAGE or against AGEs themselves [59].

Induction of NF-κB in response to oxidative stress in turn leads to a transcriptional activation of many genes, many of which are highly relevant for inflammation, immunity and atherosclerosis. These include tumor necrosis factors (TNF-α and TNF-β), interleukins 1, 6 and 8 (IL-1, IL-6 and IL-8), interferon-γ (IFN-γ), and cell adhesion molecules [23,60,61].

It is important to note that the tethering of AGEs to the cell-surface is not enough to generate ROS and cellular activation, since the RAGE carboxy-terminal cytosolic tail, containing known signaling phosphorylation sites, kinase domains, and other activation sites, is critical for RAGE-dependent cellular activation. In fact, a truncated form of RAGE, lacking only the cytosolic tail and expressed in cells, retains the binding to various ligands identically as wild-type RAGE, but does not mediate the induction of cellular activation [55].

Triggering of inflammatory effector mechanisms (generation of cytokines and chemokines, and expression of cell adhesion molecules) mediated by the AGE–RAGE interaction involves multiple intracellular signal transduction pathways, including p21ras, MAP kinases, PI3 kinase, cdc42/rac, Jak/STAT, NAD(P)H oxidase and others [59,61–66] (Fig. 3). Each of these pathways is closely linked to AGE binding to RAGE, because blockade of the receptor with

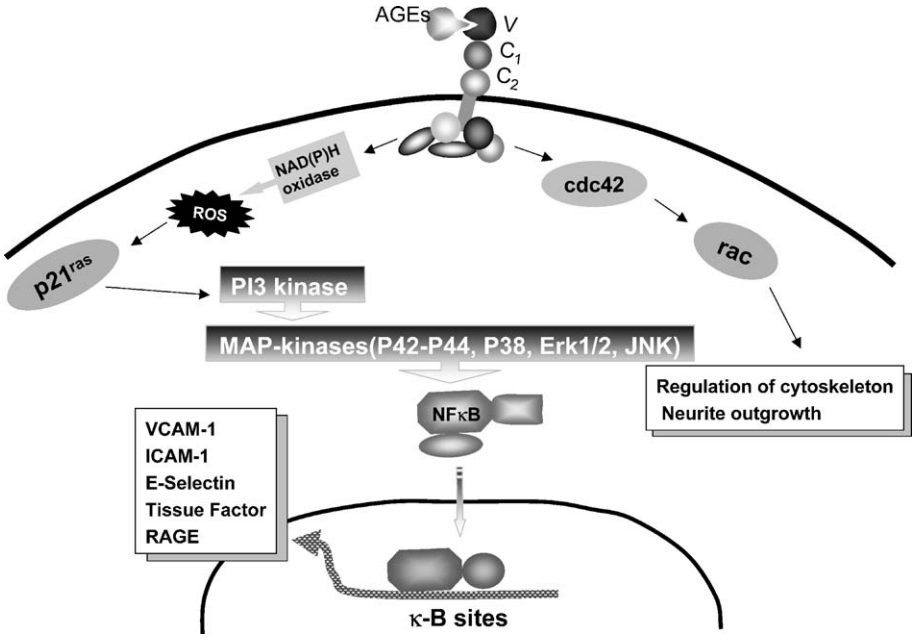

Fig. 3. Signal transduction pathways activated by RAGE–ligand interaction. The extracellular region of RAGE consists of one V-type (variable) immunoglobulin domain, followed by two C-type (constant) immunoglobulin domains, stabilized by internal disulfide bridges between cysteine residues. The V-type domain includes two putative N-linked glycation sites. In addition to the extracellular domain, RAGE displays a single putative transmembrane-spanning region and a short, highly charged cytosolic tail. Activation of RAGE by AGEs induces the increased generation of oxygen radicals by an NAD(P)H oxidase. Free radicals then activate a Ras-MAP kinase pathway eventually leading to the activation and nuclear translocation of NF-κB. A distinct signaling pathway (cdc42/rac) is responsible for RAGE-mediated neurite outgrowth.

either anti-RAGE IgG or excess soluble (s)RAGE prevents their activation.

5.5. Interaction of AGEs with mononuclear phagocytes

The interaction of AGEs with mononuclear phagocytes MPs has been shown to induce a phenotype of activated macrophages, manifested by the induction of platelet-derived growth factor, insulin-like growth factor-1, and pro-inflammatory cytokines, such as IL-1β and TNF-α [67–69]. In MPs, AGE–RAGE interaction prompts cell migration (chemotaxis). This is mediated by the interaction of soluble RAGE ligands (AGEs prepared in vitro or isolated from diabetic subjects, AGE-β2-microglobulin or CML-adducts) with RAGE. In contrast to the effect of soluble AGEs, immobilized AGEs, such as those found in basement membranes, slow down MP migration, a process known as ‘‘apoptaxis’’. Both chemotactic and apoptactic responses are blocked by anti-RAGE IgG or sRAGE [70,71].

In a more recent study, EN-RAGE has been utilized as a stimulus to induce chemotaxis. The induced migration of MPs has here been shown to be concentration- and RAGE-dependent. Similarly, the engagement of RAGE by EN-RAGE in cultured Bv2 cells (murine macrophages) induced production of IL-1β and TNF-α, in an NF-κB-dependent fashion [55]. On the other hand, when MPs reach a site of immobilized AGEs in the tissue, their migration is diminished, allowing them to bind to the AGE-modified surface and become activated. This could provide a mechanism for attracting and retaining MPs at sites of AGE deposition in tissues.

Recently, in humans diabetic plaque macrophages, RAGE overexpression has been associated with enhanced inflammatory reaction, cyclooxygenase-2/prostaglandin E synthase-1 expression; this effect may contribute to plaque destabilization through the induction of metalloproteinase expression [72].

5.6. Interaction of AGEs with vascular smooth muscle cells

Cultured smooth muscle cells (SMCs) in the presence of AGEs exhibit an increased proliferative activity and production of fibronectin [73]. In vivo, the effects promoting SMC growth are probably—at least in part—indirect, mediated by cytokines or growth factors induced by AGEs in the MPs. Transforming growth factor-β (TGF-β) might act as an intermediate factor in AGE-induced fibronectin production by SMC [73].

In this context, ligand–RAGE interaction in perturbed SMCs has important implications for the biology of restenosis [74].

5.7. Interactions of AGEs with vascular endothelium: alterations of vascular permeability and of adhesive properties

Because of its unique position and numerous properties, the vascular endothelium is particularly important in the

regulation of permeability, the maintenance of blood fluidity, the regulation of vascular growth and tone, and metabolism of hormones and vasoactive mediators. The endothelium is exposed to AGEs localized on circulating proteins or cells (for example, diabetic red blood cells), as well as those present in the underlying subendothelial matrix. Receptors for AGEs have been found on the endothelial cell surface, and mediate both the uptake and transcytosis of AGEs, as well as the internal signal transduction. AGE–RAGE interaction causes alteration of barrier function that has been documented with an increased permeability of endothelial cells incubated with AGEs and increased transit of macromolecules through the endothelial monolayer. The increase in permeability is accompanied by alterations of the physical integrity of the endothelium, as shown by the destruction of organized actin structures and alterations of cellular morphology [75,76].

It has also been demonstrated that AGEs determine alterations of endothelial anti-hemostatic functions in vitro, as shown by a reduction of thrombomodulin expression and the concomitant induction of tissue factor expression [75,77]. The induction of tissue factor and the reduction in thrombomodulin activity change the dynamic endothelial properties with regard to hemostasis from those of an anticoagulant to those of a procoagulant surface.

Binding of AGEs to endothelial RAGE also results in the depletion of cellular antioxidant defense mechanisms (e.g. glutathione, vitamin C) [63] and the generation of ROS [58] (Fig. 2). As a consequence of the increased cellular oxidative stress, NF-κB activation occurs, thus promoting the expression of NF-κB-regulated genes including, in addition to the procoagulant tissue factor, adhesion molecules, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) [23,75,77]; this past may prime diabetic vasculature towards enhanced interaction with circulating monocytes [78,79]. Also the incubation of endothelial cells with EN-RAGE or S100B causes VCAM-1 induction, in a RAGE-dependent manner, as confirmed by the inhibitory effect of anti-RAGE IgG or sRAGE [55].

5.8. Alterations of endothelium-dependent vasodilatation

AGEs linked to the vascular matrix can chemically interfere with the bioavailability of nitric oxide (NO), an important regulator of vascular tone inducing SMC relaxation [80,81]. AGEs, when added to NO in vitro, block NO activity in a concentration-dependent manner. Studies on animal models with experimentally induced diabetes demonstrate that an alteration of endothelium-dependent dilatation occurs quickly, within 2 months, from diabetes induction [80]. Presumably, the inactivation of NO occurs through a direct reaction of the NO radical with other free radicals that are formed during the reactions of advanced glycation. In parallel, AGEs induce the expression of the potent vasoconstrictor endothelin-1 changing endothelial function towards vasoconstriction [82].

6. Effects of AGEs in experimental animals

The first evidence of the direct pathogenetic role of AGEs—independent of hyperglycemia and other possible contributory factors occurring in diabetes—has been obtained in animal models, namely healthy euglycemic rats treated with AGEs. With such treatment, tissue deposition of AGEs occurs, and this is accompanied by various changes in vascular function, including alterations of permeability, subendothelial sequestration of monocytes, and decreased sensitivity to vasodilatory agents [83,84]. Nondiabetic rabbits treated for long time with ‘‘physiological’’ amounts of AGEs also manifest AGE deposition in aortic tissue and the expression of adhesion molecules such as VCAM-1 and ICAM-1 [85].

To dissect the contribution of RAGE–ligand interaction in the pathogenesis of diabetic vasculopathy, an acute animal model of diabetes-associated hyperpermeability was tested first, using reagents blocking the receptor itself or blocking the access of ligands to RAGE, by administering the decoy protein soluble (s)RAGE [76]. Rats rendered diabetic with streptozotocin, after 9–11 weeks of diabetes showed increased vascular permeability in multiple organs, especially the intestine, the skin and the kidneys. Tissue permeability was here normalized with RAGE blockade, using either sRAGE or monospecific antibodies.

7. Lessons from genetically engineered animal models

A major contribution to the understanding of the development of accelerated diabetic macrovascular disease has come from the availability of murine models of atherosclerosis. Since mice are inherently resistant to the development of atherosclerosis, in part due to their high plasma levels of high-density lipoproteins (HDL), strains genetically susceptible to atherosclerosis have been used. In mice deficient in apolipoprotein (apo) E, which develop spontaneous atherosclerosis on a normal chow diet, induction of diabetes with streptozotocin was associated with an approximately five-fold increase in mean atherosclerotic lesion area at the aortic sinus after 6 weeks of diabetes compared to euglycemic apoE null mice of the same age [86]. The administration of sRAGE in diabetic apoE-null animals suppressed accelerated diabetic atherosclerosis. Interestingly, in this contest, euglycemic animals receiving sRAGE also demonstrated a trend towards diminished atherosclerosis compared to vehicle-treated animals [86,87]. Taken together, these findings strongly suggest that AGEs, the formation of which certainly occurs even in an euglycemic environment due to normal levels of glucose and to oxidant stress, or plasma levels of other ligands, such as EN-RAGE, may be involved in the initiation and progression of atherosclerosis, at least in part, in a RAGE-dependent manner [87].

It is well-established that smooth muscle cell proliferation, migration and the expression of extracellular matrix

proteins and matrix metalloproteinases contribute to neointimal formation upon vascular injury. It has been demonstrated that wild-type C57BL/6 mice undergoing arterial endothelial denudation displayed a striking upregulation of RAGE in the injured vessel, particularly in activated smooth muscle cells of the expanding neointima [74]. Blockade of RAGE, by soluble truncated receptor or antibodies, or the absence of RAGE in homozygous RAGE null mice, resulted in significantly decreased neointimal expansion after arterial injury and decreased smooth muscle cell proliferation, migration and expression of extracellular matrix proteins [74]. A critical role for smooth muscle cell RAGE signaling was demonstrated in mice bearing a transgene encoding a RAGE cytosolic tail deletion mutant, specifically in smooth muscle cells, driven by the SM22a promoter. Upon arterial injury, neointimal expansion was strikingly suppressed compared to that observed in wild-type littermates. These data highlight key roles for RAGE in modulating smooth muscle cell properties after injury [74,88].

Recently, it has been shown that RAGE functions also as an endothelial adhesion receptor promoting leukocyte recruitment by a direct interaction of RAGE with the leukocyte beta2-integrin Mac-189. In an animal model of thioglycollate-induced acute peritonitis, leukocyte recruitment was significantly impaired in RAGE-deficient mice as opposed to wild-type mice. In diabetic wild-type mice enhanced leukocyte recruitment to the inflamed peritoneum was observed compared with nondiabetic wild-type mice; this phenomenon was abrogated in the presence of soluble RAGE, and was absent in diabetic RAGE-deficient mice [89]. RAGE–Mac-1 interaction defines a novel pathway of leukocyte recruitment relevant in inflammatory disorders associated with increased RAGE expression.

8. Possible therapeutic interventions on AGE formation, AGE cross-linking and AGE–RAGE interaction

Pharmacologic agents that specifically inhibit AGE formation have allowed the investigation of the role of AGEs in the development of diabetic complications in animal models [90]. The hydrazine compound aminoguanidine was the first AGE inhibitor discovered [34]. Aminoguanidine and other similar AGE inhibitors are thought to function as nucleophilic traps for reactive carbonyl intermediates in the formation of AGEs [91], rather than interfering with Amadori's products on proteins. Although the results in animal models of diabetic complications, demonstrating a decrease of AGE accumulation [92], are encouraging, the place of these AGE inhibitors must be better defined by clinical studies [93]. Clinical trials with aminoguanidine have also shown a trend toward reduced renal dysfunction in human diabetic subjects with advanced nephropathy [93].

While aminoguanidine prevents AGE formation, it will probably not be effective in patients with a long history of disease that already resulted in extensive tissue AGE accumulation. The need to remove irreversibly bound AGEs from connective tissues and matrix components has led to the development of AGE-cleaving agents [94]. Studies in animal models and preliminary clinical trials have shown the ability of the AGE-inhibitor pimagedine and the cross-link breaker ALT-711 to reduce the severity of pathological lesions associated with AGEs [90,95,96].

In clinical settings characterized by enhanced cellular activation or oxidative stress, such as diabetes, the expression of RAGE is enhanced, and the prolonged exposure of AGEs to RAGE-expressing cells determines a chronic state of cellular activation [97]. In contrast with other response systems in which a negative feedback stopping cellular activation is soon established, the binding of several ligands to RAGE gives rise—as highlighted above—to a series of cellular mechanisms, including the activation of NF-κB, leading to an increased expression of the receptor itself, and therefore perpetuating cellular perturbations. This suggests that interference with the vicious cycle established by RAGE–ligand interaction would interrupt cellular activation, and consequently lead to an improvement of various chronic disorders [33,98]. Nevertheless, to definitely establish the role of RAGE-dependent mechanisms in the pathogenesis of chronic disorders, more experiments in animal models in which the expression of RAGE has been genetically manipulated are needed. These would provide insights into potential physiological functions of this molecule and its ligands, and also serve the purpose of developing low molecular weight RAGE inhibitors.

9. Conclusions

The experimental evidence gathered so far unequivocally demonstrates that AGEs can alter vessel wall homeostasis in a pro-atherogenic fashion through multiple mechanisms: alterations of extracellular matrix permeability, release of inflammatory cytokines and growth factors, alterations of antithrombotic properties of the endothelium and of the ability of the vessel wall to modulate vascular tone, and the increased expression of adhesion molecules and chemokines on vascular cells. Once initiated, a state of chronic vascular inflammation ensues, sustained by the migration and activation of inflammatory cells—mostly mononuclear phagocytes and T cells—that infiltrate the altered vessel wall. These processes thus trigger a cycle of ongoing cellular injury and vascular dysfunction, in part through the release of inflammatory peptides, such as S100/calgranulins and amphoterin, which are also ligands of RAGE. The pivotal role of RAGE in these processes highlights this ligand-receptor axis as a logical and attractive candidate for therapeutic intervention to limit diabetic vascular damage and its long-term consequences.

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