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EDITORIAL

Do advanced glycation end products contribute to the development of long-term diabetic complications?

Introduction

Epidemiological, clinical and experimental evidence indicates that long-term diabetic complications are related to the extent and duration of metabolic derangement. Among the various mechanisms of hyperglycaemic injury, a major role has been attributed to the accumulation of advanced glycation end products (AGEs) in target tissues of complications; as a matter of fact, several observations indicate (1) a significant correlation between AGE levels and the presence and extent of micro- and macrovascular disease in both human patients and animal models of diabetes; (2) the induction of diabetes-like vascular pathology by administration of exogenous AGEs; and (3) a beneficial effect of agent blocking AGE formation and/or cross-linking in experimental animals [1]. These findings have prompted the initiation of phase II and III trials using these agents in patients with diabetes, suffering from micro- and macrovascular complications. However, the two randomized double blind phase III trials conducted with aminoguanidine (pimagedine) in patients with type 1 and 2 diabetes, respectively, failed to achieve statistical significance for the primary endpoints and showed significant side effects of the drug [1]. More recently, the AGE cross-link breaker ALT-711 (alagebrium) has been tested in phase II trials whose clinical endpoints were diastolic heart failure, reduced arterial compliance and systolic hypertension, i.e. the cardiovascular abnormalities closely related to the heart and vessel wall stiffening caused by AGE-dependent collagen cross-linking [2]. These studies showed that this agent is safe and quite effective in the treatment of these AGE-related abnormalities [2], though improvement was less marked than that observed in experimental animals, as for other ''pathogenic'' interventions. This might be due to inadequate trial design, particularly in terms of stage of the disease at which intervention is started. However, the limited benefit provided by alagebrium to human patients has raised the question on whether the available data are sufficient to confirm that AGEs/ALEs contribute significantly to the development of long-term diabetic complications. To answer this question, we should consider the chemical nature of AGEs, the mechanisms leading to AGE formation and those underlying their potential injurious effects.

AGEs are heterogeneous, partly unidentified compounds derived from protein glycation and including also precursors such as the Amadori rearrangement products and the dicarbonyls glyoxal (GO) and methylglyoxal (MGO) [1]. They are distinguished in pre-melanoidins, which include the precursors and the non-coloured, non-cross-linking and non-fluorescent AGEs, such as pyrraline and carboxymethyllysine (CML, the predominant AGE found in vivo), and melanoidins, which include the coloured, cross-linking AGEs, both fluorescent, such as pentosidine, and non-fluorescent, such as the lysine dimers derived from dicarbonyls (Table 1). Similar to AGEs are the advanced lipoxidation end products (ALEs) originating from metal-catalyzed oxidation of polyunsaturated fatty acids with formation of lipid hydroperoxides; the latter are further processed to form epoxyhydroxy, ketohydroxy, and cyclic derivatives which, in turn, may either decompose to aldehydes, ketones or alcohols, or condense to polymers. Lipid peroxidation products form ALEs by reacting with protein to generate labile or stable adducts or cross-links in protein, some of which may be coloured or fluorescent [1]. Some substances, such as CML, can originate from both sugars and lipid oxidation products and were called either advanced glycation or lipoxidation end products (EAGLEs) [1].

AGEs and ALEs are formed endogenously due to one or more of the following mechanisms [3]: (1) enhanced carbohydrate and lipid substrate availability, which favours generation of the reversible Schiff base and the more stable Amadori product, ultimately resulting in AGE formation; (2) increased oxidative metabolism, which causes autoxidation of glucose (autoxidative glycation) or Amadori products (glycoxidation) via formation of dicarbonyl compounds such

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Table 1 Classification of advanced glycation end products (AGEs)

Class	Characteristics	Subclass	Structures
Pre-melanoidins	Uncoloured Non-fluorescent	AGE-precursors	Glyoxal (GO) Methylglyoxal (MGO)
	Non-cross-linking	AGEs	Pyrraline Carboxyethyllysine (CEL) Carboxymethyllysine (CML)
Melanoidins	Coloured (yellow to brown) Fluorescent or non-fluorescent	Fluorescent	Pentosidine Crossline
	Cross-linking	Non-fluorescent	Glyoxal lysine dimer (GOLD) Methylglyoxal lysine dimer (MOLD) Alkyl formyl glycosyl pyrroles (AFGP) Arginine—lysine imidazole (ALI)

as GO; and (3) increased non-oxidative metabolism with accumulation of reducing sugars other than glucose, which results in the formation of the AGE-precursor MGO. AGEs may also derive from food or even tobacco, though the contribution of these exogenous sources is debated, since only a minor part of AGEs is absorbed from the intestinal mucosa [4]. Finally, tissue deposition of both endogenous and exogenous AGEs is favoured by impaired detoxification and reduced kidney clearance [3]. Thus, though the human organism is equipped with very effective and redundant defence mechanisms against these compounds, the detoxifying systems are overwhelmed under conditions of increased AGE formation or reduced AGE clearance, and also due to consumption of cofactors of detoxifying enzymes (Fig. 1). These conditions include not only diabetes but also aging, dyslipidemia, central obesity, hypertension, rheumatic and immune diseases and renal failure.

AGEs/ALES are known to display both direct, physicochemical, and indirect, biological effects. Direct effects consist of trapping and cross-linking of macromolecules which may alter their function. Indirect effects are mediated by cell surface receptors, which have a dual function, since they are involved in AGE removal, but also in AGE-induced cell activation, via (1) receptor-mediated generation of reactive oxygen species (ROS) through both mitochondrial and cytosolic pathways; (2) ROS-dependent triggering of pro-inflammatory signals causing mitogen-activated protein kinase-dependent activation of transcription factors such as nuclear factor κB and activating protein-1; and (3) consequent modulation of gene expression of several pro-inflammatory and pro-fibrotic cytokines (Fig. 2).

Several AGE-binding proteins have been identified so far [5], including the receptor for AGE (RAGE), oligosaccharyltransferase (OST)-48 or AGE-R1, 80K-H or AGE-R2, galectin-3 or AGE-R3, and the scavenger receptors (SRs) AI/AII and BI and CD36 (Fig. 2), with the classical AGE receptors and SRs sharing also the ability of binding modified lipoproteins. This redundancy seems to imply functional specificity of AGE receptors (and SRs), with RAGE predominantly involved in cell activation (signalling receptor) and OST48/AGE-R1, galectin-3/AGE-R3 and SRs mediating mainly AGE removal (endocytic receptors). In addition, since AGE receptors (and SRs) are multi-ligand, multi-functional receptors, the final net effect of AGE binding to these surface molecules is dependent on the balance between their signalling and endocytic activity, but also on whether other receptor ligands are present or other functions are stimulated. In fact, RAGE binds also calgranulins, amyloid peptide and amphoterin, thus participating in the modulation of inflammation, amyloid deposition and tumour growth [6]. Conversely, galectin-3 binds several cell surface/extracellular and intracellular proteins, thus regulating cell-to-cell and cell-to matrix contacts, inflammation, cell cycle and mRNA splicing activity [7]. Finally, SRs bind modified lipoproteins, apoptotic cells and infectious pathogens, thus modulating lipid influx/efflux and intracellular metabolism, removal of cell debris and innate immunity [8]. Studies using transgenic animals (and soluble RAGE, which competes with cell surface RAGE for AGE binding) have demonstrated that RAGE promotes atherogenesis, nephropathy and neuropathy in diabetic animals [6], whereas galectin-3 (and AGE-R1) exerts a prevailing protective role as an AGE receptor, at least in kidney disease [7]. Moreover, SR-AI and II and CD36 showed a predominant pro-atherogenic role, whereas SR-BI was found to exert a protective function towards lesion development [8].

This extreme heterogeneity of AGE/ALE structures, receptors and disease conditions in which AGEs/ALEs accumulate and are believed to play a pathogenetic role, raises several additional questions.

Are AGEs/ALEs all the same in terms of toxicity?

Not all the AGEs/ALEs could be toxic for the human body, since some of them might be inert and others, particularly the AGE precursors, could be highly reactive and potentially harmful for vascular and other target tissues. This would require examination of the effect of each of these compounds separately, though the CML, the predominant AGE found in vivo, has been shown to bind RAGE and trigger RAGE-mediated signalling leading to cell activation [9].

Are current assay techniques adequate to quantify AGE/ALE circulating and tissue levels?

The currently used immunoassays for AGE/ALE quantification yield only semi-quantitative results. In addition, the

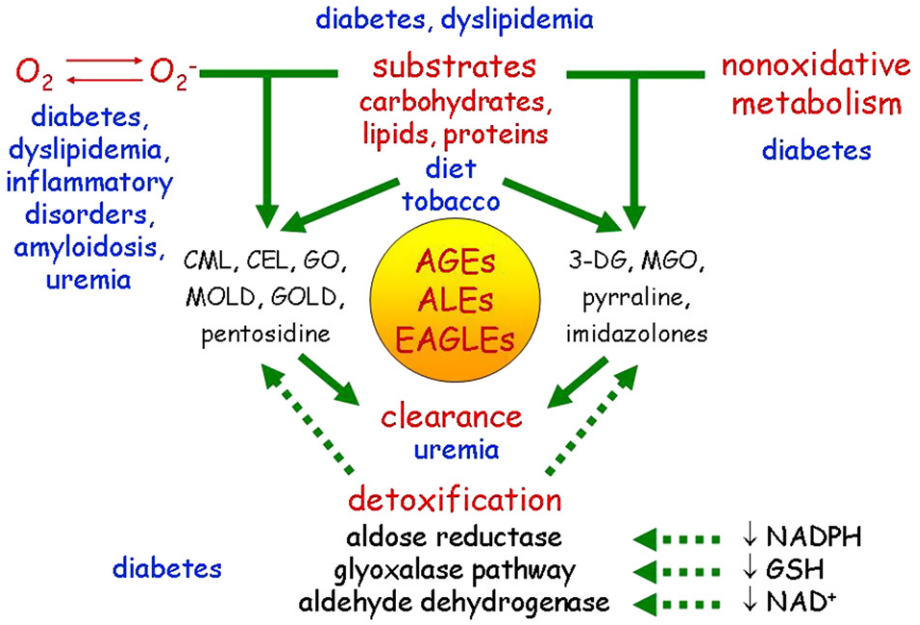

Figure 1 Sources of carbonyl formation and pathological conditions associated with increased accumulation of these compounds. O_2^- = superoxide; CML = carboxymethyllysine; CEL = carboxyethyllysine; GO = glyoxal; MOLD = methylglyoxal lysine dimer; GOLD = glyoxal lysine dimer; 3-DG = 3-deoxyglucosone; MGO = methylglyoxal; NADPH = reduced nicotinamide adenine dinucleotide phosphate; GSH = reduced glutathione; NAD^+ = oxidized nicotinamide adenine dinucleotide; AGEs = advanced glycation end products; ALEs = advanced lipoxidation end products; EAGLEs = either advanced glycation or lipoxidation end products.

specificity of the antibodies is often difficult to define with certainty and no monospecific antibodies are commercially available. Furthermore, proteins used to block non-specific binding in immunoassays may also contain AGE epitopes and thus interact with the antibody. Finally, because of steric constraints, not all AGE epitopes on the protein may be available for interaction with the antibody and factors competing for the reaction between the anti-AGE antibody and its antigen, including anti-AGE auto-antibodies and, possibly, complement, were demonstrated in plasma. Thus, quantitative analytical techniques, such as LC—MS/MS [10], should be preferably used to obtain more reliable data in

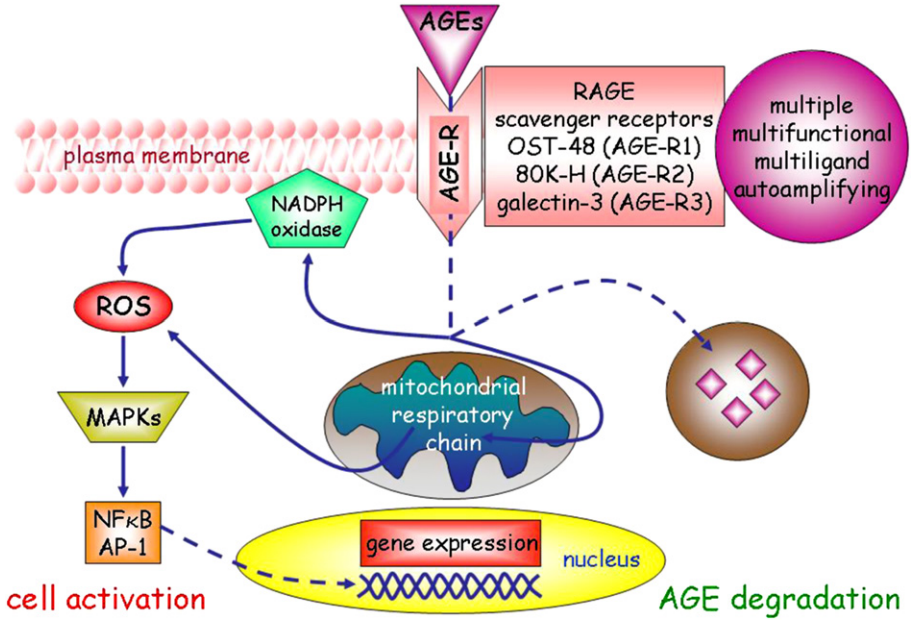

Figure 2 The AGE/AGE- (and scavenger-) receptor pathway. AGEs = advanced glycation end products; AGE-R = AGE-receptor; RAGE = receptor for AGEs; OST-48 = oligosaccharyltransferase-48; NADPH = reduced nicotinamide adenine dinucleotide phosphate; ROS = reactive oxygen species; MAPKs = mitogen-activated protein kinases; NFκB = nuclear factor κB; AP-1 = activating protein-1.

order to evaluate the role of each AGE/ALE in the development of long-term diabetic complications.

Are AGEs/ALEs involved in the pathogenesis of all the disease conditions in which their circulating and tissue levels are increased?

The demonstration that AGE/ALE levels are increased in several disease conditions in addition to diabetes would imply that, if AGEs/ALEs are truly involved in the athogenesis of diabetic complications, they should also participate in the development of vascular disease complicating these other pathologies, with the increased circulating and tissue AGEs/AGEs representing a sort of common denominator of all these conditions. This view is consistent with the experimental evidence that vascular disease associated with diabetes (and also with other normoglycaemic conditions characterized by increased AGE/ALE levels) is prevented by agents blocking AGE formation and/or cross-linking [11].

Are AGE/ALE levels the sole determinant of their toxicity?

The redundancy and functional heterogeneity of AGE receptors suggest that AGE/ALE-induced toxicity is dependent also on which receptor pathway(s) are predominantly involved in the removal of these compounds and not simply related to their circulating and tissue levels. Though the available data on the association between polymorphic allele variants of genes coding for the AGE receptors and diabetic complications are inconclusive, the evidence that circulating levels of soluble RAGE are inversely related to the presence and severity of these diabetic sequelae (and also of other conditions associated with AGE/ALE accumulation) [12] militates in favour of a central pathogenic role of AGE receptors.

Are AGEs/ALEs the damaging effectors or do they simply mask and/or mark the true injurious mechanism(s)?

This strict relationship between AGEs/ALEs and oxidative stress prompted the hypothesis that these compounds, rather than the damaging effectors, might simply be markers of oxidation, which would be the true injurious mechanism. In fact, agents such as aminoguanidine or alagebrium, in addition to reducing AGE/ALE accumulation, have additional properties, including the metal-chelating and antioxidant effect, and ROS-targeted interventions are capable of preventing disease conditions associated with increased AGE/ALE accumulation. However, the intracellular generation of ROS, which in turn promotes AGE/ALE formation via both non-oxidative and oxidative mechanisms, is a direct consequence of AGE/ALE binding to their receptors [13]. Thus, both AGEs/ALEs and ROS participate in a vicious cycle which plays a central role in the pathogenesis of diabetic complications [12], and a blockade of either one could be effective in interrupting the cascade of biochemical events leading to tissue injury.

Taken together, the available data indicate that AGEs/ALEs are certainly involved in the pathogenesis of diabetic complications by participating in a vicious cycle involving oxidative stress and AGE receptor-mediated pathways. However, further studies concerning individual AGE/ALE structures and using more quantitative techniques are necessary to conclusively demonstrate this postulate. In conclusion, effective and safe therapeutic strategies aimed at reduction of AGE formation and cross-linking and/or interference with AGE receptor-mediated events might be beneficial in retarding or preventing diabetic complications.

References

[1] Ahmed N. Advanced glycation endproducts — role in pathology of diabetic complications. Diabetes Res Clin Pract 2005;67:3—21.
[2] Bakris GL, Bank AJ, Kass DA, Neutel JM, Preston RA, Oparil S. Advanced glycation end-product cross-link breakers. A novel approach to cardiovascular pathologies related to the aging process. Am J Hypertens 2004;17:23S—30S.
[3] Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999;48:1—9.
[4] Vlassara H. Advanced glycation in health and disease: role of the modern environment. Ann N Y Acad Sci 2005;1043:452—60.
[5] Thornalley PJ. Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. Cell Mol Biol 1998;44:1013—23.
[6] Yan SF, Barile GR, D'Agati V, Du Yan S, Ramasamy R, Schmidt AM. The biology of RAGE and its ligands: uncovering mechanisms at the heart of diabetes and its complications. Curr Diab Rep 2007;7:146—53.
[7] Iacobini C, Amadio L, Oddi G, Ricci C, Barsotti P, Missori S, et al. Role of galectin-3 in diabetic nephropathy. J Am Soc Nephrol 2003;14:S264—70.
[8] Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis. beyond lipid uptake. Arterioscler Thromb Vasc Biol 2006; 26:1702—11.
[9] Kislinger T, Fu C, Huber B, Qu W, Taguchi A, Du Yan S, et al. N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. J Biol Chem 1999;274:31740—9.
[10] Thornalley PJ. Measurement of protein glycation, glycated peptides, and glycation free adducts. Perit Dial Int 2005;25:522—33.
[11] Susic D. Cross-link breakers as a new therapeutic approach to cardiovascular disease. Biochem Soc Trans 2007;35:853—6.
[12] Yamagishi S, Matsui T, Nakamura K. Kinetics, role and therapeutic implications of endogenous soluble form of receptor for advanced glycation end products (sRAGE) in diabetes. Curr Drug Targets 2007;8:1138—43.
[13] Tan AL, Forbes JM, Cooper ME. AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol 2007;27:130—43.

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