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Forum: Role of Oxidation in Atherosclerosis

GLYCOXIDATION AND LIPOXIDATION IN ATHEROGENESIS

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Abstract—Atherosclerosis may be viewed as an age-related disease initiated by nonenzymatic, chemical reactions in a biological system. The peroxidation of lipids in lipoproteins in the vascular wall leads to local production of reactive carbonyl species that mediate recruitment of macrophages, cellular activation and proliferation, and chemical modification of vascular proteins by advanced lipoxidation end-products (ALEs). The ALEs and their precursors affect the structure and function of the vascular wall, setting the stage for atherogenesis. The increased risk for atherosclerosis in diabetes may result from additional carbonyl production from carbohydrates and additional chemical modification of proteins by advanced glycation end-products (AGEs). Failure to maintain homeostasis and the increase in oxidizable substrate (lipid) alone, rather than oxidative stress, is the likely source of the increase in reactive carbonyl precursors and the resultant ALEs and AGEs in atherosclerosis. Nucleophilic AGE-inhibitors, such as aminoguanidine and pyridoxamine, which trap reactive carbonyls and inhibit the formation of AGEs in diabetes, also trap bioactive lipids and precursors of ALEs in atherosclerosis. These drugs should be effective in retarding the development of atherosclerosis, even in nondiabetic patients. © 2000 Elsevier Science Inc.

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INTRODUCTION

Atherosclerosis is a chronic disease initiated by retention of lipoproteins in the vascular wall. The deposition of lipoproteins may derive from a number of sources, including alterations in lipoprotein structure and/or size, such as the association of small, dense LDL with development of macrovascular disease [1]. Lipoproteins may also aggregate spontaneously in response to shear

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For over 20 years, Drs. Baynes and Thorpe, a husband and wife team, have studied the role of carbohydrates, lipids, and oxidative stress in the chemical modification and cross-linking of proteins during aging and in diabetes and atherosclerosis.

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stresses or enzymatic modification [2]. It is not the accumulated lipids or aggregated lipoproteins per se, but the peroxidation of lipids in the vascular wall that is implicated in atherogenesis. Bioactive lipids formed during oxidation of lipoproteins induce cellular activation and proliferation [3,4]. They also degrade to reactive carbonyl compounds that react with proteins, forming advanced lipoxidation end-products (ALEs), the residual evidence of exposure of proteins to lipid peroxidation reactions [5–7]. ALEs affect not only the structure and recognition of tissue proteins, including lipoproteins, but also modify the charge, hydrophobicity, and elasticity (cross-linking) of the extracellular matrix of the vascular wall. The additional accumulation of advanced glycation end-products (AGEs) in tissue proteins [8] is a likely source of the increased risk for macrovascular disease in diabetes.

The purpose of this article is to discuss the evidence that the chemistry of lipids and carbohydrates, expressed in the form of advanced lipoxidation and glycoxidation reactions, is a major factor in the pathogenesis of atherosclerosis. From the viewpoint that aging is the result of chronic, cumulative chemical modification of proteins

and other biomolecules, we propose that atherosclerosis is an age-related disease characterized by accelerated lipid peroxidation and lipoxidative aging of proteins in the vascular wall. We will focus on the role of increased substrate concentration in blood (hyperlipidemia) in the pathogenesis of atherosclerosis and on the mechanisms by which diabetes, which is characterized by disturbances in both carbohydrate and lipid metabolism, is an independent risk factor for the development of vascular disease.

CHEMICAL DAMAGE DURING AGING

Life is a highly regulated biological process, and aging may be viewed as the counterprocess by which random chemical reactions gradually degrade the performance of the biological system. Death, in this context, results from the gradual dominance of chemical entropy over the biological order of the living system. Were it not for sophisticated genomic control mechanisms and expenditure of energy, our biological system would collapse at any time and, under sterile conditions, purely chemical reactions of its components would mediate our biblical "return to dust." In the meantime, during the course of living, whether we subscribe to genetic, error-catastrophe, or chemical hypotheses on aging, stochastic chemical damage, the measurable evidence of aging—products of "the other side of metabolism" [9]—accumulates in all tissues with age. The tables cited later in this article list a range of nonenzymatic modifications of proteins associated with aging and age-related diseases. This chemical damage promotes damage—cell death causes an increase in oxidative stress—and local cycles of stress and damage may accelerate the aging of tissues.

Long-lived organisms have better resources for slowing down the chemistry of aging, but chemical damage to informational and functional components of tissues is a hallmark of aging, even in healthy individuals. The evidence for time-dependent chemical aging of biological systems is obvious at many levels, including structural rearrangements and deletions of DNA, chemical modification and racemization of amino acids in proteins, increases in the brown color, fluorescence and cross-linking of collagens and crystallins, and accumulation of lipofuscin in the cytoplasm. It is not necessary to argue that the rate of chemical damage increases with age, but only that the damage accumulates with age and contributes to pathology. However, some of the increase in age-related damage may result from a decrease in the rate of protein turnover with age.

Age-related diseases are characterized by an increase in chemical damage to proteins in specific organ systems. This is most obvious in the case of chronic diseases, such as atherosclerosis and diabetes. These diseases are asso-ciated with increased chemical modification of proteins at sites of pathology, by both ALEs and AGEs in atherosclerosis [5,7,10], vascular injury [11], and diabetes [8]. The level of ALEs, AGEs, and other oxidation products is a useful index of the progression of disease; however, the known products of lipoxidation and glycoxidation reactions may be viewed as the "tip of the iceberg." The majority of chemical modifications and cross-links appear to be labile to the acid hydrolysis procedures commonly for their isolation from protein.

SUBSTRATE-INDUCED AGING

Measurements of plasma metabolite, enzyme, or protein concentrations are useful for the diagnosis and management of disease. An increase in plasma glucose or lipid concentration is also indicative of, or a risk factor for, chronic age-related diseases. Hyperlipidemia is a significant risk factor for development of macrovascular disease, and hyperglycemia is the major risk factor for a range of diabetic complications. It is irrelevant whether the increase in plasma lipids and/or carbohydrates is the result of genetic, environmental, or age-related factors. Hyperlipidemia, regardless of its origin, from a genetic defect such as a mutation in a lipoprotein or its receptor, or secondary to an endocrine disease such as diabetes, or the result of dietary excesses, is still a risk factor for atherosclerosis. Similarly, increased blood glucose is a risk factor for diabetic complications, whether it results from insulin deficiency, insulin resistance, or even deliberate overexpression of gluconeogenic enzymes in animal models [12]. Increases in substrate concentrations in plasma or other tissues may be a cause or effect of perturbations in metabolic homeostasis. However, from the viewpoint of chemical hypotheses on aging, the excess of carbohydrate or lipids in blood, that is, substrate excess, is the problem per se, and a risk factor for age-related disease. Our ability to maintain homeostasis and to control substrate concentration in plasma declines with age, consistent with a role for substrate excess in both aging and age-related diseases.

ALES, AGES, AND BAGLES

In atherosclerosis, the process of lipoxidation, the chemical modification of protein by products of lipid peroxidation reactions, begins with the formation of lipid peroxides containing conjugated dienes. Lipid peroxides may be formed by enzymatic (lipoxygenase) or nonenzymatic (metal-catalyzed) mechanisms, or by both, such as the enzymatic formation of hydrogen peroxide, followed by metal-catalyzed peroxidation. The role of free metal ions in lipid peroxidation in vivo is disputed, but it

Table 1. ALEs Found in Oxidized Lipoproteins or Atherosclerotic Lesions[a]

Adducts and crosslinks	Structure	Reference
Malondialdehyde-lysine (MDA-Lys)	Schiff base adduct	[5,72]
Hydroxynonenal-lysine (HNE-Lys)	Michael adduct	[5,72]
Hydroxynonenal-lysine	Pyrrole	[16]
N$^\epsilon$-(3-formyl-3,4-dehydropiperidino)lysine (FDP-Lys)	Piperidine	[18]
Levuglandin adducts	Pyrrole	[7]
N$^\epsilon$-(hexanoyl)lysine	Amide	[19]
2-hydroxy-3-imino-1,2-dihydropyrrol	Dilysine pyrrole	[17]

[a] All of these compounds have been detected in lesions by imunohistochemical techniques; MDA-Lys and HNE-Lys have been quantified in oxidized lipoproteins by chemical assays.

is important to recognize that lipid peroxidation does not require metal ions. Pure arachidonic acid, for example, autoxidizes spontaneously and rapidly under air; metal ions catalyze the process, but are not essential. Enzymatic processes may be especially important in atherogenesis, since myeloperoxidase (MPO), lipoxygenase, and nitric oxide synthase—all of which are present in lesions—may also catalyze lipoprotein oxidation.

Decomposition of lipid peroxides initiates chain reactions that produce literally dozens of reactive carbonyl compounds, including linear and cyclic aliphatic aldehydes and ketones, and their hydroxy-, keto- and α,β-unsaturated derivatives [13,14]. Among them, the most studied carbonyl intermediates are malondialdehyde (MDA), 4-hydroxynonenal (HNE), and acrolein, which react with cysteine, histidine, and lysine residues in protein, generating characteristic ALEs (Table 1) [15]. Other ALEs, including pyrroles [16,17], piperidines [18], levuglandin adducts [7], and hexanoic acid amides [19], have also been described. Although MDA and HNE adducts are increased in atherosclerotic lesions, there is no evidence that these or other structurally characterized ALEs increase normally in tissue proteins with age. This may result from the chemical reversibility of Schiff base and Michael adducts of MDA or HNE with protein. The residual aldehyde or enol functional groups of these ALEs may also proceed to form other products, including protein cross-links and fluorescent products analogous to AGEs. Lipofuscin, the poorly characterized, fluorescent age pigment that accumulates in postmitotic cells is also considered to be the residual debris from lipoxidation reactions [20].

Carbohydrates react with amino groups in proteins via their carbonyl groups, and then undergo rearrangements involving ene-diol or eneaminol intermediates to form Amadori or Heyns adducts [21,22]. Unlike lipoxidation chemistry, reactions of carbohydrates with protein do not require oxidation chemistry. Amadori adducts formed under anaerobic conditions rearrange, also under anaerobic conditions, to more reactive dicarbonyl compounds such as 1- and 3-deoxyglucosone (DGs), which may react further to yield glyoxal (GO) or methylglyoxal

(MGO) by reverse aldol reactions. Phosphorylated intermediates in anaerobic metabolism of carbohydrates, such as triose phosphates and fructose 3-phosphate, also eliminate phosphate to form MGO [23] and 3DG [24], respectively. These dicarbonyl compounds react with lysine and arginine residues in protein under anaerobic conditions to form AGEs, including pyrraline [25], imidazolones [26,27], argpyrimidine [28,29], and cross-link structures (Table 2). Although oxidation is not required for modification of protein by carbohydrates, oxidation reactions accelerate the chemical modification of proteins by hexoses and are essential for chemical modification of proteins by ascorbate [8]. Some AGEs may be formed under completely anaerobic conditions (e.g., pyrraline and hydroimidazolones), but the aromatic, fluorescent AGEs, pentosidine [30,31], crosslines [32], and vesperlysines [33], require oxygen for their formation from glucose, DGs, or ascorbate, and are therefore termed glycoxidation products. All three of these glycoxidation products increase in tissue proteins in diabetes and during normal aging [30,32,34]. All of the compounds listed in Table 2 are increased in tissue proteins in diabetes, but none have been measured in atherosclerotic lesions. The overall contribution of AGEs, compared to ALEs, to the chemical modification and cross-linking of proteins in the nondiabetic vascular wall is still uncertain.

Table 2. AGEs Found in Tissue Proteins[a]

Adducts and crosslinks	Structure	Reference
Pyrraline	Lysine pyrrole carboxaldehyde	[25]
3-DG-hydroimidazolone	Arginine-3DG imidazolone adduct	[27]
Pentosidine	Arginine-lysine crosslink	[30,31,35]
Crosslines	Dilysine crosslink	[32]
Vesperlysines	Dilysine crosslink	[33,34]

[a] Only pentosidine has been quantified using instrumental methods; the other AGEs have been measured using immunohistochemical techniques. All of these compounds have been detected in tissue protein from control and diabetic patients, but effects of atherosclerosis on their formation are unknown.

Table 3. EAGLEs Found in Tissue Protein[a]

Adducts and crosslinks	Structure	Reference
N^ϵ-(carboxymethyl)lysine (CML)	N-carboxyalkyl adduct	[35, 36, 37]
N^ϵ-(carboxyethyl)lysine (CEL)	N-carboxyalkyl adduct	[36]
5-Methyl-hydroimidazolone	Arginine-MGO imidazolone	[26, 29]
argpyrimidine	Arginine-pyrimidine adduct	[28, 29]
Glyoxal-lysine dimer (GOLD, imidazolysine)	Imidazolium salt	[38, 39]
Methylglyoxal-lysine dimer (MOLD, methylimidazolysine)	Imidazolium sale	[38, 39]

[a] CML, CEL, GOLD, and MOLD have been quantified in tissue proteins using instrumental techniques. Based on immunohistochemical measurements, CML is increased in atherosclerotic lesions, independent of diabetes. Arginine-MGO imidazolone and argpyrimidine have been detected in tissues by immunohistochemistry, and are increased in diabetes.

In addition to ALEs and AGEs, there is a group of compounds that we refer to as EAGLEs: either advanced glycation or lipoxidation end-products (Table 3), so named because they may be formed from either carbohydrates or lipids. N^ϵ-(carboxymethyl)lysine (CML) [35] and N^ϵ-(carboxyethyl)lysine (CEL) [36] are, on a molar basis, the major nonenzymatic chemical modifications that have been measured in tissue proteins. They were originally described as products of oxidative cleavage of Schiff base or Amadori adducts to protein, but later also identified as products formed on protein during oxidation of polyunsaturated fatty acids [37]. GO, glycolaldehyde, and MGO react with proteins to form CML and CEL, respectively. The di-lysine imidazolium salts, glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD) [38,39] are also formed from GO and MGO, but have been detected thus far only in glycoxidation reactions. GOLD and MOLD are major cross-links in glycoxidized proteins in vitro and in vivo, present at 50- to 100-fold higher concentrations than pentosidine, crosslines, or vesperlysines [34,40]. CML (and probably CEL) is increased in the vascular wall in atherosclerosis [10] and diabetes [40], but its accumulation in vascular collagen in atherosclerosis, independent of diabetes, suggests that CML is formed primarily by lipoxidation reactions [41]. GOLD and MOLD also increase normally in long-lived tissue proteins (collagens and crystallins) with age, and in plasma proteins in diabetes [39], but their concentration in the vascular wall in atherosclerosis has not been measured.

In addition to the lysine derivatives, EAGLEs also include GO and MGO adducts to arginine residues in protein. The MGO imidazolone [26] and argpyrimidine [28,29] have been detected immunologically in tissue proteins, but none of the arginine derivatives have been detected in tissue proteins by chemical, chromatographic, or mass spectrometric assays. The kinetics of reversibility of the imidazolone adducts or their conversion to other products is not well studied. Overall, however, the major EAGLEs, CML, CEL, GOLD, and MOLD are quantitatively the most prominent biomarkers of nonenzymatic modification and cross-linking of protein in aging and disease.

The origin of EAGLEs, from lipids vs. carbohydrates, is perhaps an oversimplification of the true situation. There is new evidence that CML is also formed during MPO-catalyzed oxidation of amino acids, especially serine [42]. Dityrosine is also formed in MPO-catalyzed reactions and, like CML, levels of dityrosine increase in the vascular wall with the progression of atherosclerosis [43]. The quantitative contribution of MPO and amino acids to overall protein modification is unknown. Because of the lower concentrations of amino acids in tissues and plasma, compared to lipids and glucose, and their greater stability to oxidative degradation, their role as a source of reactive carbonyl compounds is likely to be limited to regions of high oxidative stress. This, of course, includes the vascular wall during atherogenesis, but lipids seem more likely sources of EAGLEs in atherosclerotic lesions. There are a number of relatively stable amino acid oxidation products that also accumulate in tissue proteins in aging and disease (Table 4), and some of these products appear to cluster in atherosclerotic plaque, along with ALEs, AGEs, and EAGLEs [31,44]. They are formed from reactive oxygen species (ROS) such as HOCl, ONOOH and H_2O_2, and may be more indicative of late-stage inflammatory processes, rather than disease-specific biomarkers.

Table 4. Amino Acid Oxidation Products Found in Tissue Protein[a]

Stable oxidation products	Possible sources	Reference
Chlorotyrosine	MPO (HOCl)	[43,44]
Nitrotyrosine	Nitric oxide synthase (ONOOH)	[33,44]
Methionine sulfoxide	H_2O_2, ONOOH, other ROS	[73]
o-Tyrosine	metal-ion catalysis	[43,73]
Dityrosine	MPO, peroxidase	[43,44]

[a] Chlorotyrosine, nitrotyrosine, and dityrosine are increased in atherosclerotic lesions. Methionine sulfoxide and o-tyrosine increase with age in collagen, but are not known to be affected by diabetes or atherosclerosis.

PATHOGENIC SIGNIFICANCE OF ALES, AGES, AND EAGLES

The toxic species formed during nonenzymatic chemistry include not only the end-products—ALEs, AGEs, and EAGLEs—but also the soluble, reactive intermediates in their formation. Precursors, such as MGO, MDA, and HNE are cytotoxic [15], and isoprostanes formed during lipid peroxidation mimic many of the biological properties of natural prostanoids [4]. ALE-containing lipoproteins may be either stimulatory [45] or toxic [46] to cells. AGE-proteins (glycoxidized proteins) induce oxidative stress responses in cells bearing RAGE (receptor for AGE), including vascular endothelial, smooth muscle cells, and macrophages [47,48]. Identification of the actual species initiating the biological response to modified proteins is difficult because of their heterogeneity and their presence at only trace levels in proteins. CML, CEL, and MDA-Lys and HNE-Lys, for example, have been detected in atherosclerotic plaque, but the total extent of lysine modification by all of these compounds is less than 1% of the lysine residues in plaque proteins, even at advanced age or advanced stages of disease. Fluorescent AGE cross-links (Table 2) are present at much lower concentrations and are more likely to be buried between, rather than on the surface of, proteins. Despite their presence in low concentrations in proteins, there is growing evidence that protein-bound ALEs, AGEs, and EAGLEs are mediators of stress responses and tissue damage. CML and CEL, for example, may bind transition metal ions in a catalytically active form, providing a mechanism for local propagation of oxidative stress and damage in tissues [49]. MGO, and probably other dicarbonyl species, may also react with adjacent lysine residues in proteins, forming redox active species that generate ROS and catalyze autoxidation reaction [50]. In addition, there is evidence that glycated lipoproteins are not only more atherogenic, but also more susceptible to oxidative modification, and that age-dependent increases in glycoxidation of collagen may promote the retention of proteins, including lipoproteins, in the vascular wall (see reviews; [51,52]), contributing to the atherogenic environment. Regardless of mechanism, the retention of lipoproteins in the vasculature would remove also them from normal pathways of protein turnover, so that they would accumulate AGEs in the vascular wall.

In addition to RAGE, other receptors in the endothelium, such as galectin-3 [53], also recognize AGE-proteins, while macrophage scavenger receptors recognize both AGE-proteins and oxidized lipoproteins containing ALEs [54]. The combination of RAGE, galectin-3, and scavenger receptors in the vascular wall suggests an active defense against the accumulation of modified proteins, but, when weakened or chronically challenged by AGE- and ALE-proteins, these receptors may also contribute to the development of atherosclerosis. In support of this argument, injection of soluble RAGE in an atherosclerosis-prone, diabetic mouse inhibited lesion formation, presumably by inhibiting the binding of circulating AGE-proteins to the vascular wall [55]. We would argue, according to this model, that the chronic excess of substrate—lipids in atherosclerosis, carbohydrate in diabetes—leads to excessive production of reactive carbonyl intermediates, thence to increased chemical modification of proteins and chronic saturation of receptors, such as RAGE, which mediate the atherogenic process. Even in the absence of receptors, ALE-proteins with lipophilic surfaces resulting from the presence of levuglandin adducts [7] or hexanoic acid amides [19], may bind to plasma membranes and induce stress responses. Thus, the chemical modification of proteins by lipids and carbohydrates cannot be viewed as an innocuous process—it may be a critical step in the pathogenesis of vascular disease.

Therapeutic approaches inhibiting nonenzymatic modification of proteins

Based on principles of biochemical regulation of metabolic pathways, it is most efficient to control a sequence of events at the earliest possible stage, at the rate-limiting or first committed step in a pathway. Similarly, in the management of atherosclerosis, therapy begins with an effort to decrease plasma cholesterol and triglycerides, just as the treatment of diabetes begins with efforts to achieve normoglycemia. For many patients, however, dietary and drug management of hyperlipidemia and hyperglycemia are of limited efficacy, indicating the need for drugs that inhibit the potential damage from these substrates, even when they remain in excess concentration in the body. At this time, however, there are no drugs approved specifically for the treatment of atherosclerosis or diabetic complications, which were designed to work in the presence of persistent hyperlipidemia or hyperglycemia. The presence of ALEs and other oxidation products in arterial plaque suggests that antioxidant defenses are inadequate, but there is little evidence that antioxidant therapy is an effective intervention in humans [56], except in individuals with vitamin deficiencies. Indeed, the oxidation of plaque lipoproteins and formation of ALEs and other oxidation products appear to occur despite fairly high levels of vitamins C and E in the vascular wall [57].

As an alternative to reduction in substrate concentration or oxidative stress, a number of drug candidates have been introduced for inhibiting the formation of AGEs in diabetes. Aminoguanidine (AG) is the proto-

type AGE inhibitor. In the original study [58], it was shown to inhibit the cross-linking and fluorescence of aortic collagen in diabetic rats, and in a series of later studies was shown to retard the development of the full range of diabetic complications, including nephropathy, neuropathy, retinopathy, and vasculopathy (see reviews; [59,60]). These therapeutic effects were achieved without an effect on hyperglycemia. AG and all other AGE inhibitors evaluated to date, including OPB-9195 [61], diaminophenazine [62], tenilsetam [63], and pyridoxamine (PM) [64], are nucleophilic compounds, designed to trap reactive carbonyl intermediates in AGE formation. In recent studies we observed that PM, a post-Amadori inhibitor of AGE formation [64,65], partially inhibited the increase in CML and CEL, cross-linking and fluorescence in skin collagen of streptozotocin-diabetic rats, and significantly retarded the development of diabetic nephropathy, assessed by measurement of both plasma creatinine and urinary albumin concentrations. AG and PM also caused a significant correction of hypercholesterolemia and hypertriglyceridemia in the diabetic rats, consistent with effects of AG on dyslipidemia in a short-term clinical trial in humans [66]. Although there are no equivalent drugs specifically designed for inhibiting formation of lipid derived ALEs in atherosclerosis, it is clear that AGE inhibitors have significant effects on dyslipidemia in diabetes [65,66]. In the cholesterol-fed rabbit model, AG also retarded the development of atherosclerosis, independent of diabetes [67], suggesting that AGE inhibitors have a more general role in inhibiting the AGEs, ALEs, and EAGLEs; this is consistent with the general role of reactive carbonyls in formation of all these products. Finally, it is worth noting that AG also appears to retard cardiovascular pathology, even during normal aging [68], in the absence of atherosclerosis or diabetes.

The mechanism of action of AGE inhibitors is complex. Both AG and PM, for example, are potent inhibitors of nitric oxide synthase and copper-dependent oxidases involved in amine and amino acid metabolism. AG also has profound effects on lipid peroxidation in vitro, being both catalytic and inhibitory, depending on concentration [69]. More to the point, however, we have recently observed that PM traps intermediates in lipid peroxidation and prevents the modification of lysine residues and formation of CML, CEL, MDA-Lys, and HNE-Lys during copper-catalyzed oxidation of LDL in vitro. Studies on the formation of AGEs, ALEs, and EAGLEs in obese, hyperlipidemic Zucker fa/fa rats may provide insight into the role of lipids in chemical modification of proteins and development of macrovascular disease in the nondiabetic state. Eventually, we hope to understand how carbonyl traps, such as AG and PM, affect plasma cholesterol and triglyceride concentrations,

without affecting hyperglycemia. One possibility is that lipid peroxidation and lipoxidation exacerbate dyslipidemia and that inhibition of the former may ameliorate the latter, providing a potential inroad for treatment of atherosclerosis.

CONCLUSIONS

Considered from the viewpoint of chemical hypotheses on aging and age-related pathologies, atherosclerosis and diabetes are closely related diseases. Abnormalities in lipid concentration and metabolism are associated with insulin resistance [70], an early stage in type 2 diabetes, while diabetes is an independent risk factor for both micro- macro-vascular disease [71]. In this brief overview, we have considered atherosclerosis as an age-related disease whose pathology results, in part, from an excess of substrate (peroxidizable lipids) in the circulation and in vascular lesions. We propose that deposition and oxidation of lipoproteins in the vascular wall leads to generation of reactive carbonyl intermediates, then to chronic chemical modification and cross-linking of proteins. We argue that these chemical processes are significant contributors to atherogenesis, and that this chemistry is accelerated in diabetes, the result of further increase in carbohydrate precursors and increased chemical modification of proteins by both carbohydrates and lipids. We also propose that AGE inhibitors, such as AG and PM, which are designed to intercept the chemical modification of proteins by carbohydrate-derived carbonyl compounds, will also inhibit the chemical modification of proteins during lipid peroxidation reactions, and will prove useful in the treatment of atherosclerosis.

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ABBREVIATIONS

AG—aminoguanidine

AGE—advanced glycation end-product

ALE—advanced lipoxidation end-product

CML—N^ε-(carboxymethyl)lysine

CEL—N^ε-(carboxyethyl)lysine

3DG—3-deoxyglucosone

EAGLE—either advanced glycation or lipoxidation end-products

GO—glyoxal

GOLD—the dilysine-imidazolium salt, glyoxal-lysine dimer

HNE—4-hydroxynonenal

MDA—malondialdehyde

MGO—methylglyoxal

MOLD—the dilysine-imidazolium salt, methylglyoxall-ysine dimer

PM—pyridoxamine

ROS—reactive oxygen species