1. Introduction

“We age with the senescence of our blood vessels,” the saying goes, and indeed the blood vessels are intimately related to aging. Blood vessels include arteries and veins; however, it is the arteries that change in relation to age. Arteriosclerosis develops essentially through aging, and is exacerbated by risk factors such as hypertension and dyslipidemia.

Arteriosclerosis is a pathological disease concept which refers to arterial lesions characterized by intimal thickening, stiffening, and remodeling of the arterial walls. These are classified as three different lesions: (1) atherosclerosis, characterized by an accumulation of extracellular lipids in the deep layer of the intima, known as atheroma, (2) arteriolosclerosis, causing intimal thickening, hyalinosis and/or stenosis of the small arteries and arterioles, and (3) Monckeberg’s medial calcific sclerosis, characterized by laminar or annular calcification in the tunica media of muscular-type arteries. Among these, atherosclerosis is the most critical arterial lesion because of leading to lifestyle diseases, including myocardial infarction and stroke. This paper discusses the role of advanced glycation end-products (AGEs) for the progression of atherosclerosis.

2. Atherosclerosis (Fig. 1)

According to the paper of Crawford 1), atherosclerosis is defined as “the widely prevalent arterial lesion characterized by patchy thickening of the intima, the thickenings, comprising an accumulation of fat and layers of collagen-like fibers, both of which are present in widely varying proportion.” The arterial wall thickens with age, and the thickened intima comprises fibrocellular tissues. The microscopically and chemically detectable lipids are soon deposited in the extracellular matrix of the intima. Extracellular lipids are modified by oxidation and incorporated by macrophages to form foam cells. This results in the formation of “fatty streaks,” a slightly elevated lesion with yellow streaked appearance upon gross observation, which is considered the initial lesion of atherosclerosis. Cytokines and growth factors are released by infiltrating macrophages and T lymphocytes, and stimulate the growth and production of the collagen molecules of smooth muscle cells. Smooth muscle cells also transform to foam cells in the intima. Foam cells derived from smooth muscle cells are susceptible to apoptosis 2,3). Extracellular lipids originated from dead foam cells generate a lipid-rich core containing necrotic tissues and cholesterol in the deep layer of thickened intima (atheroma), which contributes to the progression of atherosclerosis from initial to advanced lesion. An advanced lesion with a thick fibrous cap overlying the atheroma causes the narrowing of the lumen in the coronary artery, and is considered an underlying cause of stable angina pectoris, being termed a “stable plaque.” Inflammation and
growth of atheroma in this plaque make the fibrous cap thin, leading to the formation of an “unstable plaque,” which contributes to unstable angina pectoris. The rupture of the thin fibrous cap causes ulceration, hemorrhage, and thrombus formation, which lead to myocardial infarction and stroke 4).

3. AGES and fibrocellular intimal thickening

In humans, atherosclerosis develops on the basis of fibrocellular intimal thickening 5). There are two types of intimal thickening, including eccentric intimal thickening and diffuse intimal thickening. The former is localized exclusively in arterial bifurcations, while the latter is widely observed in the non-branching region of arteries. Eccentric intimal thickening is formed at bifurcations of the coronary and cerebral arteries from newborns. Thereafter, intimal thickness increases with age at the lateral wall of bifurcations, which is exposed to low and turbulent shear stress 6,7). In contrast, there is no such elevation of intimal thickness at the medial wall (apex) of bifurcations, which is a high and unilateral laminar shear stress region (Fig. 2, top). The intimal thickening in the lateral wall of bifurcations changes with age qualitatively as well as quantitatively (Fig. 2, bottom). The tunica intima of neonates is rich in smooth muscle cells embedded in myxoid or edematous extracellular matrices (cellular intimal thickening: C). After the age of 1 year, collagen and elastin fibers increase in the extracellular matrix of the intima, resulting in fibrocellular intimal thickening (CF). Connective tissue elements predominate at the deep layer of the tunica intima in humans of over 40 years of age. Atherosclerotic intimal thickening (C/F) develops via the deposition of lipids in extracellular spaces of fibrocellular intima. Advanced glycation end-products (AGES) of collagen protein are associated with an age-related accumulation of collagen fibers in the intima.

Fig. 1. A natural history of atherogenesis.

Fig. 2. Age-related intimal alterations.
A: Age-related changes of intimal thickness at lateral and medial walls at the bifurcation of anterior descending branch and circumflex branch of left coronary artery.
B: Age-related histological changes of tunica intima at the lateral wall of cerebral arterial bifurcation.
C: Cellular intimal thickening, CF: Fibrocellular intimal thickening, C/F: Atherosclerotic intimal thickening, F: Fibrous intimal thickening.
Collagen modified by AGEs increases with aging even in tunica intima free from atherosclerotic lesions (Fig. 3, left). The amount of pepsin-digested collagen negatively correlates with the level of AGE modification (Fig. 3, right). Thus, collagen fibers accumulate with advancing age in the tunica intima due to the age-related decrease in the susceptibility of collagen to protease. LDL is then deposited on AGE-modified collagen fibers which are accumulated at the deep layer of the tunica intima, and forms extracellular lipids (Fig. 4). Recently, Nω-carboxymethylarginine has been shown to be one of the AGE chemical structures which is formed exclusively in collagen fibers. This age-related accumulation of AGEs in the extracellular matrix has been observed not only in arteries, but also in other tissues such as ocular lens crystallin and skin.

Fig. 3. Age-related alterations of AGE level and extractability of collagen derived from lesion-free intima of human aorta.

A: Age-related change in the level of collagen-linked AGEs.
B: Correlation between the level of collagen AGEs and extractability of collagen. CLF: Collagen-linked fluorescence (U/mg); TW: Tissue weight.

Fig. 4. Localization of different AGEs and apolipoprotein B in fibrocellular intimal thickening of human aorta. Carboxymethyl lysine (CML, arrow), a glycoxidation product, is observed in a small number of macrophages. On the other hand, non-carboxymethyl lysine (Non-CML, arrow) is co-localized with apolipoprotein B (Apo-B, arrow) in extracellular matrices. HAM56: Macrophage.
4. AGEs and lipid deposition

An accumulation of lipoprotein derived from plasma precedes macrophage infiltration in thickened intima of young adults. Lipoprotein particles extracted from lesion-free intima show the same size and lipid composition as those of plasma low-density lipoprotein (LDL). Extracellular matrix proteoglycans serve as possible binding molecules to lipoproteins in the tunica intima. Glycated LDL is potent in deposition in the tunica intima due to its non-recognition by LDL receptors and LDL bound to proteoglycans is shown to have an increased susceptibility to oxidation. In addition to oxidation, LDL deposited in the tunica intima can also undergo glycation. The process of oxidation and glycation is closely linked because of the generation of oxygen species during glycation, being known as glycoxidation. Therefore, LDL deposited in the intima undergoes oxidation as well as glycoxidation. These modified LDLs are incorporated by macrophages and smooth muscle cells via the receptors for AGEs (RAGE) and various scavenger receptors, including types I and II SR-A, SR-BI, CD36 and LOX-1, expressed on the cell surface. These cells enhance the expression and release of inflammatory cytokines and adhesion molecules via PKC activation or MAP kinase activation, causing the migration and proliferation of smooth muscle cells and endothelial dysfunction. Consequently, intimal lesion consists primarily of macrophage foam cells and intimal smooth muscle cells containing lipid droplets, accompanied by infiltrating T lymphocytes, being referred to as fatty streaks. Oxidation causes an accumulation of lipid hydroperoxide, and the formation of malondialdehyde (MDA) in LDL. Glycoxidation results in the formation of carboxymethyl lysine (CML) in LDL. These products accumulate in atheroma seen in deep layer of advanced lesion. In contrast, pyrraline, one of the oxidation-independent products, is observed in the upper layer of this lesion, but is not present in atheroma. This indicates that oxidation and glycoxidation of LDL is related intimately to atherogenesis.

5. AGEs and plaque rupture

Plaque rupture is the direct cause of myocardial infarction and stroke, and a critical event for morbidity and mortality from atherosclerosis. Inflammatory processes play an important role for the pathogenesis of plaque vulnerability and rupture. Rupture-prone, vulnerable plaques usually contain many inflammatory cells, including macrophages and lymphocytes, which accumulate in the overlying fibrous cap and the transitional region where the plaque rises from lesion-free area, being known as the shoulder of the plaque. Secreted cytokines and adhesion molecules, including MCP-1, CD18, ICAM1 and VCAM1, contribute to the accumulation of inflammatory cells in the plaques. IL1-β, TNFα, and other cytokines stimulate macrophages and smooth muscle cells to produce and release proteolytic enzymes, such as matrix metalloproteases (MMPs). MMPs cause collagen loss in the fibrous cap and shoulder of the plaques, and promote their rupture, resulting in the direct contact of blood coagulation factors to tissue factor, a potent activator of coagulation cascade, expressed in the plaques. In coronary arteries, approximately 80% of ruptured plaques break at the shoulder region of these plaques. Overexpression of MMPs-1,3,9 and
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Fig. 6. Atheromatous plaques demonstrate co-localization of malondialdehyde (A, MDA, *) and carboxymethyl lysine (B, CML, *) in atheroma in deep layer of the intima. In contrast, pyrraline (C, Pyrraline, *) is found in the extracellular matrix of upper layer of the intima. I: Tunica intima

Fig. 7. Distribution of the maximal principal stress under the intraluminal pressure of 10-16 kPa and a longitudinal stretch of 1.07 (Fig. 7). Concentration of stress is found at the shoulder of the plaque (Arrows).

infiltration of T lymphocytes and macrophages/foam cells are observed in the shoulder of the plaques. Finite element analysis shows the concentration of maximum principal stress in the shoulder of the plaque for the pressure range of 10-16 kPa under a longitudinal stretch of 1.07 (Fig. 7). Recently, various biomarkers, including CRP, myeloperoxidase (MPO), PAPP-A, and soluble CD40 ligand, are proposed for plaque destabilization and rupture. MPO is expressed in macrophages and neutrophils that infiltrate into vulnerable and ruptured plaques. CD40-ligand and lysophosphatidylcholine, which usually exist in human atheromatous plaques, stimulate MPO-containing macrophages to produce hypochlorous acid (HOCl), a specific MPO-derived oxygen species. It is proposed that AGEs, including CML and GA-pyridine, are generated by a HOCl-serine system, which is mediated by MPO originated from activated macrophages and neutrophils. CML is detected at rupture sites of vulnerable plaque which are infiltrated by neutrophils (Fig. 8). MPO is reported to be expressed in captured particulate debris obtained from patients with carotid artery stenosis who underwent carotid artery angioplasty and stenting. In addition, CML contents in stent debris are significantly greater in symptomatic carotid stenosis patients than asymptomatic patients. Thus, neutrophils infiltrating into unstable and ruptured plaques may contribute to CML formation through the MPO-HOCl-serine system.
6. Conclusion

Atherosclerosis is a multifactorial lesion which is mediated by various mechanisms. Various AGEs show different localization in atherosclerotic plaques and have different roles in atherogenesis. AGEs can be generated in vivo by a variety of enzymatic and non-enzymatic pathways, some of which are mediated by inflammation and cell metabolism. Future research may elaborate the AGE profiles which are useful for the diagnosis and prognosis of atherosclerotic lesions.

Conflict of interest statement:

The authors declare no financial or other conflicts of interest in the writing of this paper.
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