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Review Article

Oxysterols and 4-hydroxy-2-nonenal contribute to atherosclerotic plaque destabilization

Simona Gargiulo, Gabriella Testa, Paola Gamba, Erica Staurenghi, Giuseppe Poli, Gabriella Leonarduzzi*

Department of Clinical and Biological Sciences, University of Torino, San Luigi Hospital, 10043 Orbassano, Torino, Italy

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ABSTRACT

A growing bulk of evidence suggests that cholesterol oxidation products, known as oxysterols, and 4-hydroxy-2-nonenal (HNE), the major proatherogenic components of oxidized low density lipoproteins (oxLDLs), significantly contribute to atherosclerotic plaque progression and destabilization, with eventual plaque rupture. These oxidized lipids are involved in various key steps of this complex process, mainly thanks to their ability to induce inflammation, oxidative stress, and apoptosis. This review summarizes the current knowledge of the effects induced by these compounds on vascular cells, after their accumulation in the arterial wall and in the atherosclerotic plaque.

1. Introduction

Atherosclerosis is a multifactorial and degenerative disease affecting large- and medium-sized arteries, which is characterized by chronic inflammation, oxidative stress, and by blood flow that is altered in certain areas of the vascular wall [1,2]. The lesions primarily develop and progress at the arterial wall, mediated by interactions among cytokines, growth factors and vasoregulatory molecules; these mediators regulate the function of cells intrinsic to the arterial wall and extracellular matrix (ECM) as well as blood cells and plasma constituents. Development of atherosclerotic lesions is preceded by impaired vascular endothelium function, mainly caused by oxidative stress, inflammation [1,3], and endoplasmic reticulum (ER) stress [4], all conditions that are induced by the factors promoting and accelerating atherosclerosis. Vascular endothelium dysfunction is also linked to cellular senescence [5]. The intercellular cross-talk that occurs among smooth muscle cells (SMCs), macrophages, endothelial cells (ECs) and leukocytes leads to a fibroproliferative response. During this response, the ECM plays a key role in plaque formation and progression, providing the structural integrity of the plaque itself, as well as contributing to cell migration and proliferation, and finally thrombosis. A stable atherosclerotic plaque is characterized by a thick and solid fibrous cap, where large amounts of ECM are deposited, and a small central lipid core. The strength of the fibrous cap depends on a dynamic balance between collagen synthesis and degradation. Conversely, an atherosclerotic plaque at high risk of rupture contains a large lipid core where, in addition to extracellular lipid deposition and debris, ECM degradation is enhanced, leading to increased plaque fragility; moreover, the lipid core is covered with a thin fibrous cap affected by ongoing inflammation and neovascularity [6,7].

Among the intrinsic and extrinsic factors that can trigger plaque progression, with subsequent vulnerability and rupture, key roles are played by lipid components, inflammation, cell death, and fibrous cap weakening, as well as hemodynamic stress and circumferential shear stresses [8].

One of the major risk factors of atherosclerosis is hypercholesterolemia, which promotes the accumulation of oxidatively modified low-density lipoproteins (oxLDLs) in the arterial wall, promoting endothe-

Abbreviations: α-EPOX, 5α,6α-epoxide; β-EPOX, 5β,6β-epoxide; 22-OH, 22-hydroxycholesterol; 24S-OH, 24S-hydroxycholesterol; 25-OH, 25-hydroxycholesterol; 27-OH, 27-hydroxycholesterol; 7α-OH, 7α-hydroxycholesterol; 7β-OH, 7β-hydroxycholesterol; 7-K, 7-ketocholesterol; AP-1, activator protein-1; COX-2, cyclooxygenase-2; ECs, endothelial cells; ECM, extracellular matrix; EDR, endothelium-dependent relaxation; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; HNE, 4-hydroxy-2-nonenal; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LC3-II, light chain 3-II; 5-LO, 5-lipoxygenase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; MMPs, matrix metalloproteinases; NAC, N-acetylcysteine; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; Nrf2, nuclear factor E2-related factor 2; oxLDLs, oxidized low density lipoproteins; PDGFR, platelet-derived growth factor receptor; PDI, protein disulfide isomerase; PGE_2, prostaglandin E_2; PI3K, phosphoinositide 3-kinase; PK, protein kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; SMCs, smooth muscle cells; TGF-β1, transforming growth factor-β1; TIMPs, tissue inhibitors of MMPs; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α; Triol, cholesterol-3β,5α,6β-triol; TRX, thioredoxin; UPR, unfolded protein response; VCAM-1, vascular cell adhesion molecule-1

* Corresponding author.
E-mail address: gabriella.leonarduzzi@unito.it (G. Leonarduzzi).

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lial cell dysfunction and, in turn, inflammatory response, with leukocyte invasion of the wall [9]. The development of vascular inflammation entails continuous recruitment of inflammatory and immune cells from the blood into the sub-intimal space, as a consequence of the inflammation-dependent increase in endothelial permeability; cell recruitment is favored by up-regulation of endothelial adhesion molecules, chemokines, cytokines, and growth factors. Once in the sub-intimal space, monocytes differentiate into macrophages and take up oxLDLs through the scavenger receptors CD36 and SR-A. Unlike LDL receptors, scavenger receptors are not regulated by a negative feedback loop, so that macrophages avidly accumulate oxidized lipids, becoming foam cells, and meanwhile release a large variety of proinflammatory cytokines. Perpetuation of this process promotes a chronic inflammatory state leading to the progression and instability of the atherosclerotic plaque [10,11]. Accumulated foam cells and extracellular lipids form the lipid core that, depending on its individual characteristics and lipid components, may or may not contribute to plaque instability. It appears that large amounts of cholesteryl ester may soften the lipid core, whereas cholesterol crystals increase plaque rigidity [12]. In addition, it has been shown that a lipid fraction of human atherosclerotic plaques induces oxidative stress in mouse macrophages, and decreases high density lipoproteins' ability to trigger cholesterol efflux from macrophages [13]. Moreover, a lipid extract from human carotid plaques has been shown to increase expression of proinflammatory mediators in human THP-1 cells and macrophage-like cells. Of note, the fraction rich in cholesterol oxidation products was a major contributor to this effect [14]. These data support the hypothesis that the plaque itself is atherogenic, as plaque lipids may enhance plaque formation. Further, in the plaque, the death of foam cells, activated by inflammatory mediators, results in necrotic core formation: cell death leads to the spillage of lipids and, hence, enlargement of the soft lipid core [15,16].

Migration into the fibrous cap of numerous SMCs, which synthesize collagen, and their proliferation are crucial in determining whether the cap can maintain its thickness and structural integrity. Apoptosis of SMCs, which leads to depletion of their numbers, reduction in collagen production, and thinning of the fibrous cap, is another potential cause of plaque vulnerability: an increased number of apoptotic vascular SMCs has been found in advanced symptomatic plaques, compared with stable lesions [17–19]. Inhibition of SMC apoptosis stabilizes atherosclerotic plaques *in vivo* [20].

In addition, ongoing inflammation also plays an important role in weakening the fibrous cap [10,11]. Marked infiltration of inflammatory cells, predominantly macrophages and T-lymphocytes, has been observed at the site of plaque rupture [21]. Following activation of these inflammatory cells, various cytokines and extracellular matrix-degrading metalloproteinases (MMPs) are released. Inflammatory molecules, together with oxidative stress compounds, can regulate the expression of genes involved in collagen synthesis, as well as in the expression and activity of MMPs [10,11]. Fibrous cap disruption is markedly initiated by an excess of MMPs over their inhibitors (TIMPs: tissue inhibitors of MMPs), which are produced by macrophages and macrophage-derived foam cells, while T-lymphocytes release interferon-γ, which inhibits collagen synthesis and SMC proliferation at the fibrous cap. Together, they also activate macrophages to release MMPs. In particular, MMP-9, MMP-8 and MMP-2 are markedly upregulated in macrophages and macrophage-derived foam cells. MMPs thus weaken the fibrous cap and promote plaque rupture thrombus formation, by destroying the ECM. They promote monocyte/macrophage invasion, thereby amplifying plaque inflammation, and apoptosis of ECs, SMCs, and macrophage-derived foam cells. Moreover, MMPs promote angiogenesis, favoring the development of vasa vasorum, which leaks erythrocytes into the plaque; this contributes to the formation of a large lipid core, which is an adverse feature in plaque stability [22–24].

2. Oxidized lipoproteins and atherosclerotic plaque progression

A causative role for oxLDLs in atherosclerosis has now been established [25,26]. After crossing the endothelial barrier and accumulating in the sub-endothelium, oxLDLs are significantly involved in the initiation, formation, progression, and destabilization of the atherosclerotic plaque, as they induce inflammation and oxidative stress. They also engender endothelial activation, monocyte recruitment, macrophage differentiation, and SMC migration and proliferation at moderate concentrations, whereas at higher concentrations they are proapoptotic [27].

Concerning endothelial function, oxLDLs can induce expression of angiotensin converting enzyme and receptor AT_1R in primary human vascular ECs [28]. Of note, both the endothelium-dependent relaxation (EDR) alteration in murine aortic vascular rings [29] and the foam cell formation in human macrophages [30] induced by oxLDLs were abolished on blocking AT_1R.

It has also been demonstrated that oxLDLs modify and inhibit cellular proteins, contributing to the conformational change (misfolding) and impaired function of modified proteins. As a consequence of this effect, the regulation of pathways involved in cell homeostasis (e.g. the ubiquitin/proteasome system) [31], or of cell signaling pathways, (e.g. the platelet-derived growth factor receptor (PDGFR) pathway) [32] is disturbed or inhibited. Moreover, apoptosis of vascular cells induced by oxLDLs is associated to increases in ER stress and unfolded protein response (UPR) [33]. ER stress occurs when stress signals, such as oxidative stress, cause the accumulation of misfolded or unfolded proteins in the organelle. Under normal conditions, the UPR is then activated to restore ER homeostasis; if this response is not sufficient to contrast the stress stimuli, ER triggers apoptosis [34]. Toxic concentrations of oxLDLs may also modify protein disulfide isomerase (DPI), an ER-resident chaperone and oxidoreductase, which catalyzes the formation and rearrangement of disulfide bonds; it thus participates in protein folding. DPI modification during atherosclerotic lesion formation suggests that its activity could be inhibited, with consequent alteration to the folding of nascent proteins in the ER, and potentiation of both ER stress and apoptosis induced by oxLDLs. This leads it to contribute to plaque progression, destabilization, and rupture [35].

Several reactive molecules derive from oxidation of the LDL lipid fraction, including peroxides, hydroxides, aldehydes, oxidized phospholipids, and cholesterol oxidation products. Considerable information concerning the roles of these various components is now available [36,37]. In recent decades, cholesterol oxidation products and aldehydes have attracted the attention of various research groups, because of their close involvement in the pathogenesis of atherosclerosis.

Cholesterol oxidation products, known as oxysterols, are 27-atom carbon compounds that originate from the oxidation of cholesterol by either enzymatic or non-enzymatic mechanisms; they present one or more carbonyl, keto, hydroxyl, or epoxide groups in the sterol ring and/or in the side chain (Fig. 1). They are present in both free and esterified forms [38–40].

With regard to atherosclerosis, there is no longer any doubt that oxysterols play a pivotal role in all the various steps of atheroma formation, from endothelial dysfunction to plaque fibrosis and rupture, through vascular cell infiltration/migration, proliferation and differentiation [17,19]. Of the oxysterols, 27-hydroxycholesterol (27-OH), 7-ketocholesterol (7-K), 5α,6α-epoxide (α-EPOX), 5β,6β-epoxide (β-EPOX) and cholesterol-3β,5α,6β-triol (Triol) are the most abundant oxysterols in plasma and atherosclerotic lesions [38,41,42]. It has been observed that the oxysterols commonly found in plasma from hypercholesterolemic patients are also found in atherosclerotic plaques, and a strong, direct correlation between total oxysterols and total cholesterol have been observed in plaques [43].

Among aldehydes that are end-products of n-6-polyunsaturated fatty acid peroxidation, 4-hydroxy-2-nonenal (HNE) may contribute to progression of the atherosclerotic plaque (Fig. 2). Its potential