Cholesterol oxidation products in the progression of atherosclerosis

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Summary

Oxysterols represent a family of 27-carbon cholesterol derivatives that may be absorbed with the diet or originated endogenously. These compounds are able to modulate various signaling pathways, by this way exerting a number of biochemical effects that include promotion of chronic inflammation, fibrosis and apoptotic cell death. Cholesterol oxidation products, as with the other oxidation products of the lipid moiety of plasma low density lipoproteins (LDL), are consistently found within the characteristic lesions of atherosclerosis. Of note, the challenge of cells of the macrophage lineage with a mixture of oxysterols like that detectable in hypercholesterolemic individuals leads to a marked overexpression of various inflammation-related molecules like TGFβ1 and MCP-1, but also of CD36 scavenger receptor. The possible up-regulation of these proteins within the atherosclerotic lesion indicates the ability of biologically relevant amounts of oxysterols to attract monocytic cells into the subintimal space and to stimulate their differentiation into macrophages with eventual formation of adherent foam cells. It is very likely that oxysterols make a significant contribution to the vascular changes occurring in atherosclerosis. They may be involved, besides foam cell formation, in all other key steps of this complex process, namely endothelial cell dysfunction and adhesion of circulating blood cells, deposition of extracellular matrix, and, in the unstable plaques, matrix degradation and apoptotic death of vascular cells.

Key-words: **hypercholesterolemia, atherosclerosis, oxysterols, inflammation, atheroma.**

Introductory remarks

The relation of dietary and blood cholesterol with atherosclerosis is, since many decades, a topic of intense research and considerable debate. Cholesterol is an essential component of cellular membranes but, when arterial endothelium develops an inflammatory phenotype, it can be deposited in the artery wall, leading to the formation of atherosclerotic plaque. The mechanisms by which cholesterol contributes to the initiation and in particular to the progression of atherosclerotic lesions are nowadays only in part elucidated[1].

Among the oxidation products of LDL lipid moiety, cholesterol oxidation products, named oxysterols, are of great interest as possible reactive mediators of structural and functional changes of the vascular wall during the atherosclerotic process[2]. In fact, unlike the parent compound, a poorly reactive molecule, oxysterols are provided with strong pro-inflammatory, pro-apoptotic and pro-fibrogenic effects[3,4].

Oxysterols may derive from foodstuffs that contain cholesterol, especially after long storage and cooking, or may be formed in the body, through either enzymatic or non-enzymatic reactions. Oxidation of the side chain is an enzymatic process, while oxidation of the sterol nucleus, with the only exception of 7α-hydroxycholesterol production, is commonly a non-enzymatic one. Among side-chain cholesterol oxidized compounds, 27-hydroxycholesterol (27-OH) is the one mostly represented in the hypercolesterolemic blood[1]. Besides 7α-hydroxycholesterol (7α-OH), other oxysterols deriving from sterol nucleus oxidation are thought to have pro-atherogenic potential, namely 7-ketocholesterol (7-K), 7β-hydroxycholesterol (7β-OH), 5α,6α- and 5β,6β-epoxycholesterol (α-EPOX and β-EPOX), and cholestan-3β,5α,6β-triol (TRIOL)[3,4]. All these compounds are

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consistently recovered in the plasma of normocholesterolemic individuals in the low nanomolar range, while in hypercholesterolemic subjects they could rise up to represent 1-5% of total blood cholesterol.

As regards the effective oxysterol content of human fibrotic plaques, this could vary because of several variables like stage of lesion development, great variability of fat deposition in different plaques from the same individual, and differing sensitivity of the analytical method employed. However, despite quantitative discrepancies, all studies point to 27-OH, 7-K and 7β-OH as the most abundant oxysterols in human atheromas[5].

Nowadays, high-performance liquid chromatography (HPLC) and gas-chromatography (GC) appear as reliable techniques to characterize oxidized derivatives of cholesterol, but GC-MS is by far the most powerful one[4]. Notably, cholesterol susceptibility to air oxidation can lead to over-estimation of its oxidized derivatives in biological lipid materials. Thus, analysis has to be performed on freshly collected specimens, or, alternatively, proper storage conditions must be ensured, and several precautions must be kept during sample preparation. Among them, immediate addiction of antioxidants, such as EDTA and butylated hydroxytoluene (BHT), and oxygen-free conditions (by flowing samples with nitrogen or argon) are mandatory.

Thus, a very efficient way for cholesterol to promote and sustain the progression of atherosclerosis appears that of undergoing oxidation reactions. In fact, oxysterols have been shown to be involved in various key steps of the atherosclerotic process, from endothelial cell dysfunction up to fibrotic degeneration of the arterial wall and vulnerable plaque rupture.

Oxysterols-induced change of endothelial layer homeostasis

Perturbation of ion transport activity, with increased Ca^{++}- and Na^{+}/K^{+}-ATPase activities, was observed in rabbit aortic endothelial cells challenged with a biologically compatible mixture of oxysterols[6]. A similar mixture was demonstrated to significantly increase the arterial endothelium permeability in New Zealand white rabbits[7].

Pro-inflammatory effect on cell migration and differentiation within the atherosclerotic lesion

Cholesterol oxidation products have been shown to stimulate not only the adhesion of leukocytes to the arterial endothelium but also their transmigration to sub-intimal spaces; this is achieved through a sustained and marked up-regulation of key chemotactic cytokines, particularly interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1)[8]. IL-8 is a member of the CXC chemokine family and one of the major mediators of the inflammatory response; MCP-1 is an important member of the CC chemokine family, essentially attracts to the arterial wall and activates cells of the macrophage lineage, but also peripheral blood T lymphocytes. These two inflammatory cytokines appear as key biochemical mediators of the chronic inflammatory reactions which underlie and promote the progression of atherosclerosis.

Oxysterol-induced macrophage differentiation and foam cell formation

Expression and synthesis of the CD36 scavenger receptor represents a key event in the development of the atherosclerotic lesion because of its primary involvement in modified LDL uptake by vascular cells and in foam cell formation. The challenge of the promonocytic cell line U937 with the same oxysterol mixture that up-regulated MCP-1[8], markedly stimulated both expression and synthesis of CD36, while identical amount of non-oxidized cholesterol did not exert any effect on the scavenger's level[9]. Importantly, further incubation of oxysterol-challenged cells with oxLDL led to the formation of cells with evident phagocytic vacuoles and strongly stained with the lipid dye Nile red. The essential role of CD36 scavenger receptor in foam cell formation was proved by the prevention of the latter event when oxysterol-treated monocytic cells were incubated in the presence of anti-CD36 specific antibody[9].

Effect on deposition of interstitial fibrous matrix and on fibrous cap formation

Following endothelial activation and infiltration of monocyte/macrophages, which are essential prerequisites for fibrous cap formation, migration of smooth muscle cells (SMCs) along an endothelial to medial chemotactic gradient takes place. A large variety of molecules modulates migration and proliferation of vascular SMCs, including IL-1, TGFβ1 two cytokines whose expression and synthesis are strongly stimulated in monocytes and macrophages treated with oxysterols[4]. Moreover, the same oxysterol mixture has been shown able to up-regulate TGFβ1 in macrophagic cells, significantly stimulated proliferation of human vascular SMCs (Leonarduzzi and Robbesyn, unpublished results).

Contribution of oxysterols to vascular apoptosis and matrix degradation

During the progression of the disease, atherosclerotic lesions and the surrounding arterial wall tissue may undergo extensive modification and remodeling, mainly due to intensive fibrosis and cellular death. Apoptosis appears to be the prevalent mechanism of death, and oxysterols have been shown able to trigger it. Of interest, the simultaneous induction of anti-apoptotic signals by oxysterols and other oxidized lipids accumulating within the atheroma makes the overall process much complex. It is a matter of fact that cell survival and fibrosis are prevailing in the stable lesions, while programmed cell death and extracellular matrix degradation are highly expressed in the unstable lesions (Leonarduzzi and Gargiulo, manuscript in preparation). Updated and comprehensive reviews of the generation of pro- as well as anti-apoptotic signals by oxysterols are now available[5,10].

Finally, it should be noted that most of the toxicological studies on oxysterols have been carried out on single compounds, while in the diet, as well as in the plasma and in atherosclerotic lesions, they always occur in mixtures. Thus, additional, synergistic or even quenching effects may be expected.

Conclusions

Cholesterol, accounting for about half LDL weight, may significantly contribute in promoting monocyte/macrophage-mediated inflammation as well as ECs and SMCs functional changes in the arterial wall. By this way it might represent a primary driving force of the development of atherosclerotic lesions, provided to be at least partly oxidized.

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