Oxidative elements in the pathogenesis of atherosclerosis

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In the pathogenesis of atherosclerosis several factors concur: alteration of vascular permeability, hyperlipidemia, "endothelial activation", leukocyte adhesion, imbalance of coagulation/fibrinolysis equilibrium, altered vascular tone regulation, inflammatory and degenerative phenomena, growth factor production and release. This is the so-called response-to-injury hypothesis.

Evidence has grown in the last years that alteration in the metabolism of lipoproteins, in particular oxidative modifications of low-density lipoproteins (LDL), can play a major role in triggering and amplifying the atherosclerotic process. LDL are not *per se* atherogenic but can undergo chemical modifications, which are thought to be essential in the pathogenesis of atherosclerosis. Oxidative modifications are today considered of paramount importance.

It is well known that oxidative stress can damage biological membranes through a self-amplifying process called lipoperoxidation. Oxidative stress can initiate the process by the action of a radical species (usually a reactive oxygen species) subtracting a hydrogen to a fatty acid and generating a lipid radical, which rapidly acquires conjugate diene configuration; in the presence of oxygen, the diene binds oxygen, becoming a peroxyl radical able to subtract a hydrogen to another fatty acid; in this way the peroxyl radical becomes a peroxide and generates another lipid radical. This is how lipoperoxidation becomes a self-perpetuating process. During the lipoperoxidation process some by-products are generated through the decomposition of peroxidized lipids; many of these by-products are aldehydes, capable of diffusing and of binding covalently to amino groups, or other functional groups, of proteins; aldehydic adducts of proteins are generated, which can exhibit altered structure and function. Among these by-products, malondialdehyde and 4-hydroxynonenal have received particular attention because of their quantity and their biological effects.

LDL have been shown to be prone to a lipoperoxidation process analogous to biological membranes, with loss of polyunsaturated fatty acids, generation of lipoperoxides, consumption of vitamin E, and generation of aldehydes[1]. It has also been demonstrated that aldehydes coming from lipid decomposition are able to bind amino groups of the apolipoprotein B itself, generating aldehydic adducts[2]. Other authors have underlined the possible oxidation of cholesterol in LDL, generating several oxysterols. Such modifications induce structural and functional alterations in LDL[3].

A well defined property of modified LDL has been shown to be their liability to be recognized by the scavenger receptor of macrophages[4], which is not down-regulated; this metabolic derangement is considered one of the basic causes of lipid accumulation in the vessel wall. Moreover, oxidized LDL are chemoattractant for macrophages, but then these molecules are able to inhibit macrophages movements, probably by stimulating the endothelium to produce MPC-1. Oxidized LDL have been shown to exhibit cytotoxic properties: this can induce further endothelial or smooth muscle cell damage; they can also modulate cell functions, for example they can induce the secretion of growth factors or interleukins or the expo-

sition of membrane receptors (e.g. vascular cell adhesion molecule-1).

More recently, other possible mechanisms than reactive oxygen species-mediated phenomena have been proposed for LDL lipoperoxidation *in vivo*: peroxidase-dependent oxidation[5] and hypochlorite-dependent oxidation[6]. It is conceivable that these mechanisms can be active in the atherosclerotic lesion, where macrophages are well represented, since macrophages are a physiological source of peroxidase and hypochlorite.

Moreover, it has been demonstrated that oxidized LDL, as well as many covalently modified proteins, are immunogenic. This observation has led to the hypothesis that immunological mechanisms can be involved in the pathogenesis of the atherosclerotic plaque; indeed it is known today that lymphocytes, in particular T lymphocytes, infiltrate the atherosclerotic lesions, can be attracted by cytokines, and can interact with multiple new antigens formed *in loco* by LDL oxidation too. The role of immunological mechanisms has been underlined by the observation that apolipoprotein E deficient mice, an animal model of atherosclerosis, possess high titers of autoantibodies to malondialdehyde-lysine, which should reflect the existence of such modification *in vivo*[7]. Moreover, the presence of malondialdehyde and 4-hydroxynonenal adducts in the atherosclerotic plaques has been demonstrated with immunological methodology[7,8]; the simultaneous presence of advanced glycosylation end-products gives evidence for possible interactions between oxidation and glycation: the glycoxidation process[9]. The glycoxidation process can occur inside the LDL particle: the glycation of phospholipids is accompanied by the peroxidation of their unsaturated fatty acids.

An unresolved problem is the site of LDL oxidation. It is widely accepted that the interfaces plasma/endothelium and endothelium/subendothelial macrophages can be elective oxidation sites, particularly in condition of increased LDL exudation; metal-catalyzed lipoperoxidation, peroxidase, hypochlorite and nitric oxide radical are the probable cause of LDL oxidative modification. On the contrary, circulating LDL do not seem to be extensively oxidized.

It has anyway to be kept in mind that, while we have well standardized methods for the evaluation of glycation end-products, such as pentosidine[10], markers of long-term oxidative damage accumulation are not so easily found[11]. Methodologies are often complex and molecular knowledge incomplete. Recently, a quantification of aldehydic adducts in human native LDL failed to find 4-hydroxynonenal adducts[12], which casts some shadow on the oxidative interpretation of atherosclerosis.

A different approach to evaluate oxidative damage is considering protein peroxidation, a process which has been much less studied than lipoperoxidation in the past but is now receiving increasing attention. Starting in a manner probably similar to lipoperoxidation, it leads to the formation of oxidized amino acids, some of which are possible candidate molecular markers of oxidative damage accumulation[13]. DOPA, o- and m-tyrosine, dytirosine, 5-hydroxyleucine have been signaled as increased in human atherosclerotic plaques[14]. Another group found a 100-fold increase of dityrosine in atherosclerotic lesion LDL compared to plasma, but not elevated levels of o- and m-tyrosine: this should indicate a role for tyrosyl radical but not for metal ions in the pathogenesis of atherosclerosis[15]; the same group observed that 3-nitrotyrosine was 90-fold increased in LDL of atherosclerotic intima compared with plasma LDL; this indicates a role for nitric oxide in LDL oxidation in vascular lesions[16].

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