Editorial

Phospholipid Hydrolytic Enzymes in a ‘Cesspool’ of Arterial Intimal Lipoproteins

A Mechanism for Atherogenic Lipid Accumulation

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Most of the lipid in atherosclerotic fibrous plaques is extracellular. How does it get there? Hakala and coworkers describe in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology a pathway involving enzymatic hydrolysis of phospholipids in LDL, leading to lipoprotein aggregation and fusion and hence, to accumulation of lipid droplets. The process is enabled and enhanced in the presence of arterial proteoglycans. This extracellular pathway should be distinguished from the commonly postulated cellular pathway involving uptake of modified LDL in macrophage foam cells, which subsequently die and leave the accumulated lipid in an extracellular location.

Although macrophages and macrophage antigens can be found in the “necrotic,” lipid-rich core of plaques, several lines of evidence strongly suggest that most of the lipid derives not from dead foam cells but from the extracellular trapping and processing of LDL. The evidence can be sketched briefly as follows: (1) The earliest core regions are located deep within the intima of atherosclerotic fatty streaks (in the musculoelastic intimal sublayer) below the level of macrophage foam cells. (2) The lipid core, especially in smaller fibrous plaques, shows a markedly increased ratio of free to esterified cholesterol, whereas the hypothesis of foam cell death would predict more esterified cholesterol. (3) The fatty acyl pattern of core cholesterol esters has more linoleate than oleate, similar to the pattern of plasma lipoproteins, but quite different from the oleate predominance of lipoprotein aggregation and fusion of LDL. (4) Extracellular lipid droplets in human lesions are similar to those observed in human lesions. In a rabbit model, freeze-etch electron microscopy captured instances of particle fusion occurring in the arterial intima just 2 hours after intravenous infusion of a bolus of human LDL. In pursuing the hypothesis of lipoprotein aggregation and fusion, an essential task is to define relevant biochemical mechanisms. Early work suggested that oxidation of LDL enhances aggregation, and phospholipase C as well as mast cell proteases were shown to induce aggregation and fusion of LDL. However, the extent to which these in vitro demonstrations applied to the human arterial wall was uncertain.

Over the past 8 years, a role for sphingomyelinase in atherogenic lipid accumulation has been delineated. Schissel and coworkers found that arterial endothelial cells and macrophages secrete an enzyme capable of hydrolyzing sphingomyelin in LDL at neutral pH. This secretory sphingomyelinase originates from the same gene, mRNA, and polypeptide precursor as acid (lysosomal) sphingomyelinase but differs in its carbohydrate moieties and in the absence of secretory sphingomyelinase is induced to cause aggregation and fusion of LDL and was identified immunohistochemically in human atherosclerotic lesions. Secretory sphingomyelinase was shown to cause aggregation and fusion of LDL and was identified immunohistochemically in human atherosclerotic lesions. Secretory sphingomyelinase by human vascular endothelial cells is induced by inflammatory cytokines, and plasma sphingomyelinase activity is increased in mouse models of systemic inflammation.

Type IIa secretory nonpancreatic phospholipase A (snPLA2), as reviewed by Hurt-Camejo and Camejo, is a 14-kDa, highly basic, glycosaminoglycan-binding enzyme that hydrolyzes phospholipids at the sn-2 position. A signal sequence in its precursor polypeptide and its dependence on millimolar (extracellular) levels of calcium mark it as an extracellular enzyme. snPLA2 is implicated in several inflammatory diseases by high tissue levels in the presence of disease and by its ability to produce a set of inflammatory mediators from phospholipids. Interferon-γ induces snPLA2 in human arterial smooth muscle cells. The pres-
ence of snpPLA₂ in human atherosclerotic plaque has been demonstrated.20–22

Hakala and coworkers23 in a previous study exposed LDL to phospholipase A₂ obtained from bee venom. Aggregation and fusion of the LDL particles occurred in the presence of heparin, but only LDL aggregation occurred in the absence of heparin. In the present study, the authors have extended their findings by using human snpPLA₂ from transfected Chinese hamster ovary cells as well as human aortic proteoglycans (PGs). In the presence of PG, human snpPLA₂ induced aggregation and fusion of LDL to form larger lipid particles. These larger particles were shown to have higher binding affinity to PG than did native-size LDL. Furthermore, the total amount of LDL binding to PG was increased 3-fold by enzymatic phospholipid hydrolysis. The combination of aggregation, fusion, and binding to PGs describes a potential process of lipid accumulation from LDL in the extracellular space.¹

The activities of both phospholipid hydrolytic enzymes—sphingomyelinase and snpPLA₂—fit into the framework of the “response-to-retention” hypothesis of atherogenesis (specifically, LDL retention) proposed by Williams and Tabas.24,25 The hypothesis emphasizes LDL aggregation and binding to arterial PGs. Fusion of LDL, also part of the hypothesis, implies a transformation into larger lipid particles that are less mobile and not easily removed from the artery wall. Whether sphingomyelinase, snpPLA₂, both, or some other attack on LDL particle integrity might be crucial to such lipid deposition is not fully known. Answers may be sought partly through experiments designed to elucidate compositional shifts in atheroma phospholipids. The development of transgenic mice will also be helpful, and recently a mouse overexpressing snpPLA₂ was shown to have accelerated atherogenesis.26 Clinical studies may provide clues—for example, immunoreactive snpPLA₂ levels in blood plasma have been correlated with coronary atherosclerosis and subsequent events among 235 patients undergoing coronary angiography.²⁷

In the intima of large arteries, LDL is both abundant and stagnant. Concentrations of saline-extractable apolipoprotein B antigen, considered to represent soluble LDL, are roughly as high in the arterial intima as in blood plasma.²⁸,²⁹ This finding contrasts sharply with the concentrations of LDL at other connective tissue sites in the body, which are estimated to be one tenth of the plasma concentration on the basis of measurements in lymph. To reach such high LDL concentration in the arterial intima, the multiple mechanisms of biochemical LDL retention as reviewed by Williams and Tabas²⁴,²⁵ are supplemented by a simple physiological mechanism. Lymphatic vessels act as “sumps” to drain away excess macromolecular species accumulating in the extracellular space, but lymphatic vessels are absent from the arterial intima.³⁰ Lymphatic vessels, which operate at low pressure, presumably would collapse and fail to function at all in the arterial intima, which bears high hydrostatic pressure (a situation unique to the inner arterial wall among all connective tissues in the body). In any case, whether due to hydrostatic pressure or some other reason, lymphatic vessels do not grow into the arterial intima, leaving a tissue often hundreds of micrometers thick without effective sump action and drainage of macromolecules, including LDL. (In greatly thickened atherosclerotic intima with neovascularization at the base of a lipid core, lymphatic vessels may be present, but the damage has already been done there.)

Therefore, although LDL traverses the endothelial layer from plasma into the arterial intima at only limited rates, LDL accumulates in the intima to levels essentially as high as those in plasma. Furthermore, the residence time of LDL in the intima may be very long, perhaps weeks to months, because of limited permeability of the endothelium and of the tunica media.³⁰ High levels and long residence of LDL maximize the opportunity for enzymatic, oxidative, and other processes—including snpPLA₂ as described by Hatala et al—³¹ to disrupt the integrity of individual LDL particles, exposing hydrophobic or other sites that mediate aggregation, fusion, and matrix binding of the particles. The arterial intima is a cesspool for LDL. Sphingomyelinase and snpPLA₂ are 2 denizens of the cesspool that make it a hazardous place.

References


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