5-Lipoxygenase-Derived Leukotrienes
Mediators Also of Atherosclerotic Inflammation

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Atherosclerosis is now viewed as the outcome of hypercholesterolemia in combination with inflammation of the vessel wall; an intertwined sequence of events leads to fatty streaks, which may develop to atherosclerosis. Several recent studies have implicated lipoxygenases in this process. Mammalian lipoxygenases have two principal functions. One is to modify membranes by peroxidation reactions; 12/15-lipoxygenases (12/15-LOs, in homo 15-LO type I) is typically connected with this function. The other is to produce signaling lipid mediators which exert effects via G protein–coupled plasma membrane–bound receptors; maybe the best example is 5-lipoxygenase (5LO) and the leukotrienes. Accordingly, lipoxygenases can in principle contribute to the pathophysiology of atherosclerosis in two ways: by LDL oxidation and by biosynthesis of proinflammatory leukotrienes. The first articles indicating a role for 12/15-LO in oxidative modification of LDL appeared 10 years ago, for a recent review discussing pro- and anti-inflammatory leukotrienes. The first articles indicating a role for 12/15-LO in oxidative modification of LDL appeared around 10 years ago, for a recent review discussing pro- and anti-atherogenic effects of 12/15-LO, see the article by Funk and Cyrus. In the June issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Kühn et al present data showing that mutations in 5LO occurring in atherosclerosis-resistant CAST/CON6 mice reduces the activity of 5LO in vitro. Here, recent findings which support a role for 5LO and leukotrienes in atherosclerotic inflammation are discussed.

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The only mammalian lipoxygenase which has been structurally determined is the rabbit reticulocyte 15-lipoxygenase (a 12/15-LO). Based on this crystal structure, the 5LO structure can be modeled (Figure) as a monomeric enzyme with two domains. The catalytic domain contains iron, which is anchored by two conserved His and the C-terminal Ile. Such a 2-His-1-carboxylate facial triad is a common feature for active sites of mononuclear nonheme iron(II) enzymes. The smaller N-terminal domain is a C2-like β-barrel with the typical ligand-binding loops. Residues in these loops have been shown to bind Ca²⁺ and membrane, and Ca²⁺ can activate 5LO by inducing membrane association. When leukocytes are activated to produce leukotrienes, 5LO moves from the cytosol, or from a soluble locus inside the nucleus, to the perinuclear membrane. The clustering of cytosolic phospholipase A₂ (cPLA₂), 5LO, and permanently membrane-bound FLAP (5LO activating protein, transfers arachidonate to 5LO) is reasonable because membrane phospholipid is the source of substrate. FLAP and LTC₄ synthase are integral nuclear envelope proteins; the nuclear envelope was described as a LT biosynthetic metabolon.

When sorting out a role for leukotrienes in atherosclerosis, it is helpful to consider the criteria for a mediator of inflammation (Sir Henry Dale, 1929). The criteria can be stretched a little, so that enzymes catalyzing biosynthesis of the mediator are also included. Are the mediators (or enzymes) there, do they give any of the typical signs, and are the signs reduced when antagonizing the mediators (or enzymes) pharmaceutically? All three criteria can be judged positively when results from mouse and man are combined.

5LO is present in human atherosclerotic vessel wall (aorta, coronary arteries, carotid arteries) as demonstrated by RT-PCR and immunohistochemistry on samples from ~50 patients. Most of the 5LO was found in CD68⁺ macrophages in the vessel wall; many of these cells also carried the dendritic cell marker CD1a. In addition, most 5LO⁺ cells stained for HLA-DR, and many contained lipid. In view of these findings, cells containing 5LO and lipid were designated 5LO⁺ macrophage foam cells and 5LO⁺ DC foam cells. 5LO⁺ cells in vessel wall (mainly CD68⁺ macrophages/foam cells/DCs) were counted, the number was found to be higher in samples from more advanced coronary heart disease (AHA stages IV to V), as compared with stages II to III. For tunica media, the increase in 5LO⁺ cell number was ~5-fold, and for tunica intima ~7-fold. Interestingly, when atherosclerosis advances from stage III to stage IV, symptoms can become overt. Also, the presence of 5LO activating protein (FLAP), LTA₄ hydrolase, and LTC₄ synthase in human atherosclerotic vessel wall was indicated by RT-PCR and immunoblot (for FLAP).

Can the atherosclerotic vessel wall produce leukotrienes? One of the first articles combining the title words “leukotriene” and “atherosclerosis” (from 1988) presents such data. Human vascular tissue obtained at surgery was cut into small fragments and incubated with or without Ca²⁺ ionophore A23187 and arachidonic acid. LTB₄-immunoreactivity was present in the medium, in increased amounts after administration of arachidonate or ionophore. Boiling of the tissue as well as administration of lipoxygenase inhibitors decreased the LTB₄-immunoreactivity, in agreement with enzymatic formation, and it was pointed out that the amount of LTB₄⁺ immunoreactivity increased with the cellularity of the plaque incubated.
The involvement of LTB₄ in foam cell formation in apoE⁻²⁻ mice was evaluated using the LTB₄ receptor antagonist CP-105,696. Oral treatment for 35 days reduced both lipid accumulation and monocyte infiltration of aortic lesions, and lesion size was reduced. As for PMNL, LTB₄ is a potent chemotactic stimulus also for human monocytes, a response which CP-105,690 is expected to antagonize. Expression of adhesion molecules is important for monocyte extravasation, and it was found that CP-105,696 reduced levels of CD11b in lesions. Upregulation of the integrin CD11b (on leukocytes) which binds to ICAM-1 (on endothelium) may thus be another mechanism by which LTB₄ stimulates monocytes to enter the vessel wall. Previously, cysteinyl-leukotrienes (LTC₄ and LTD₄) were found to upregulate surface expression of P-selectin on HUVEC. P-selectin is one of the cell adhesion molecules active during leukocyte accumulation in atherosclerosis.

A genetic locus on chromosome 6 was recently found to confer resistance to atherosclerosis in the mouse strain CAST/Ei. The relevant segment of chromosome 6 was transferred to C57BL/6J, giving the congenic strain CON6, which was almost entirely resistant to atherosclerosis, also when the LDL receptor-null mutation was introduced. The gene for 5LO is on mouse chromosome 6, and it was found that CON6 mice expressed considerably reduced amounts of 5LO, as determined for bone marrow and peritoneal monocytes/macrophages. In addition, it was found that in the CON6 mouse the 5LO cDNA sequence was mutated, leading to exchanges of Ile-645 to Val and of Val-646 to Ile. To confirm these findings with the natural CAST/CON6 mutation, Mehrabian et al constructed 5LO/LDLr double knockout mice. Surprisingly, these two mutations together appeared to be incompatible with life, an intriguing finding because 5LO⁻/⁻ mice are quite healthy. Almost as surprising, there was a stronger reduction (than the expected 50%) of both 5LO mRNA and protein in the 5LO⁻/⁻/LDLr⁻/⁻ mice, and the aortic lesions in these 5LO heterozygous mice were reduced to an amazing 5% of control. Too good to be true some colleagues say, but it should be observed that the findings with CAST/CON6 and with the double knockouts are two separate experiments, giving two lines of evidence.

The present article by Kühn et al follows up on the CON6 mutation of the 5LO coding sequence (Ile-645 to Val and Val-646 to Ile, naturally occurring in CAST/Ei). Ile-645 and Val-646 reside in one of the long α-helices of the 5LO catalytic domain (see the Figure). One could visualize that mutation of these residues might affect translocations of 5LO and thus cellular 5LO activity, or the mutation could affect the catalytic properties of the 5LO enzyme. Human 5LO cDNA (94% identical to mouse 5LO) was mutated and expressed in Escherichia coli; both the double mutation I645V + V646I and the single mutations I645V or V646I were tested. For all three mutants, K_M was slightly decreased, but more strikingly V_max was reduced to less than 20% of control, and the resulting relative 5LO activities for purified enzymes in vitro ranged between 11% and 19% of control. 5LO catalyzes not only the conversion of arachidonic acid to 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE), but also the following step leading to leukotriene A₁, and the mutations diminished also the LTA₁-synthase activity. When an adjacent residue in this helix (Arg-651) was mutated to Gln, 5LO activity in cell-free assays was lost further supporting that this region of 5LO is important for activity. Thus, from the present paper it is clear that CAST/CON6 mice have a strongly reduced 5LO activity, which is due to both reduced 5LO expression, and to the inactivating mutation I645V + V646I.

5LO enzyme activity is tightly regulated, so how might 5LO in macrophages/foam cells/DCs of the vessel wall become activated? Ca²⁺ is a well-established stimulus for 5LO leading to membrane association, and signal transduction pathways leading to increased Ca²⁺ in these cells should be operative. In addition, 5LO was recently found to be phosphorylated at Ser-271 by MAPKAP kinase 2/3 downstream of p38 MAPK and these pathways were activated by cell stress. So called “chemical stress” (sodium arsenite) and osmotic stress could stimulate 5LO in PMNL, also after chelation of Ca²⁺ both extra- and intracellularly. The atherosclerotic vessel wall is often connected with oxidative stress, and there are data supporting that also this type of cell stress can activate 5LO. Thus, 5LO in a B-lymphocyte cell line (BL41E95A) was activated by hydrogen peroxide, and this activation was sensitive to the p38 MAPK inhibitor SB 203580. Several years ago, it was found that for human PMNL subjected to oxidative stress (induced by Diamide or 1-chloro-2,4-dinitrobenzene) conversion of arachidonic acid to 5(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HETE) was upregulated 5- to 10-fold. Thus it appears possible that 5LO also in cells of the vessel wall can be activated by oxidative stress, via p38 MAPK.

This editorial advocates a role for 5LO and leukotrienes in the recruitment of monocytes/macrophages into the vessel wall during pathogenesis of atherosclerosis. Can we exclude that 5LO produces lipid hydroperoxides leading to secondary
oxidation (seeding) of LDL? In a study addressing this question using different 5LO inhibitors, no support for a role of 5LO in human monocyte-mediated LDL oxidation was obtained.\textsuperscript{32} Furthermore, there are no known examples of 5LO leading to membrane modification by peroxidation. Instead, 5LO is a closely regulated enzyme which produces trienes,\textsuperscript{3} but also to lipoxins \textsuperscript{33} and 5-oxo-ETE.\textsuperscript{34} The leukotrienes are established mediators of inflammation, so why not also of atherosclerotic inflammation?

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