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Lipolytic enzymes in atherosclerosis as the potential target of inhibitors

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Abstract

It is at present generally accepted that three components—modification of lipoproteins, infection and inflammation play an important role in the evaluation of atherosclerotic lesions. Low density lipoprotein (LDL) can undergo modification and accumulate over time in the artery wall. Modified LDL particles share the ability to induce inflammatory functions of cells associated with atheroma. The present review will focus on the multifunction action of lipases and phospholipases in lipid and lipoprotein metabolism, and atherosclerosis. The development of highly specific lipolytic enzyme inhibitors not only for studying their kinetic effects but also for potential pharmacological application is discussed.

Keywords: Atherosclerosis; Lipolytic enzymes; Inhibitors

1. Introduction

Atherosclerosis is a complex process involving the interplay of genetic and environmental factors and the involvement of multiple cell types. The hallmark of atherosclerosis is the accumulation of cells containing excessive lipids (i.e. foam cells) within the arterial wall. The endothelium plays an important role in vascular blood flow and it is now apparent that endothelial disfunction contributes to the pathogenesis of atherosclerosis. The endothelial cell and subendothelial cell matrix provide a stage for several interactions that mediate lipoprotein transport, retention, and modification during atherogenesis. A later phase of lesion development involves smooth muscle cell (SMC) migration and proliferation, which is thought to be influenced by both endothelial cells and monocyte-derived macrophages [1]. Plasma lipoproteins are the major source of the lipid that accumulates in atherosclerotic lesions. Atherogenesis is also generally considered a chronic inflammatory disease [2]. Expression of cytokines and growth factors by vascular and inflammatory cells is thought to contribute to the development of the atherosclerotic lesion [3]. Recently, *Chlamydia pneumoniae* and *Helicobacter pylori* have also been reported to be pathogenic agents in the mechanism leading to atherosclerosis [4,5].

However, even if importance of three components—cholesterol, infection and inflammation is appreciated in the genesis of atherosclerosis we are still ignorant of their interrelationships and we still do not know which of the three comes first.

2. Modified LDL—trigger of atherosclerosis and inflammation

Modification of low density lipoprotein (LDL) particles appears to be a key event in the initiation of

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atherogenesis [6]. Oxidative modification, even when mild, alters the structure of the LDL particles and renders them capable of aggregation. The modified LDL particles can trigger various proinflammatory reactions mainly via lipids, such as fatty acids, lysophosphatidylcholine, oxidatively modified phospholipids, ceramide and unesterified cholesterol [6,7]. LDL particles isolated from atherosclerotic lesions and extracellular lipid droplets are relatively enriched in sphingomyelin and lysophosphatidylcholine (lysoPC).

One of the types of LDL particle modification involves lipolysis. Lipolysis of LDL phospholipids by phospholipase A_2 [8] and lipolysis of cholesterol esters by carboxyl ester lipase (CEL) [9] release fatty acids from LDL, which may lower smooth muscle cell proliferation and induce secretion of extracellular matrix capable of trapping increasing amounts of LDL [1]. Lipases that hydrolyze triglycerides in triglyceride-rich lipoproteins also have the potential to hydrolyze LDL core triglycerides, leading to the formation of modified LDL particles. Both of these lipolytic modifications lead to aggregation and/or fusion of LDL [8]. Anchoring of LDL to extracellular matrix increases the residence time of LDL in the intima, and so allows extensive modification, possibly of multiple types, to take place [6]. Cholesterol esterase-treated LDL, if taken up by macrophages, induce secretion of macrophage chemotactic protein-1 (MCP-1) by these cells, a powerful cytokine capable of inducing influx of monocytes into inflammatory tissue sites, such as atherosclerotic lesions [10]. In the human arterial intima, notably in inflamed areas, a phospholipase of secretory type, capable of hydrolysing LDL phosphatidylcholine (PC), has been found [3]. High plasma carboxyl ester lipase has been shown to correlate positively with plasma cholesterol and LDL levels, suggesting a possible role for CEL in formation and accumulation of atherogenic lipoprotein [11]. Moreover, this enzyme has been shown to be secreted by both endothelial cells and macrophages in culture and reported in human atherosclerotic aorta [12].

The findings support the view that the lipolytic enzymes play roles in the modification of LDL during atherogenesis. Since modification of LDL particles is likely to be critical for their atherogenicity, i.e. for accumulation of lipids in the arterial intima and the resulting inflammation, inhibition of LDL modification could be a therapeutic goal.

3. Lipases

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) form a large group of enzymes that catalyse hydrolysis of a wide range of carboxyl esters. Lipases are widely distributed enzymes, involved in the degradation of triacylglycerols (TAG) in a variety of tissues. Lipoprotein lipase (LpL), hepatic lipase (HL), endothelial lipase (EL) and pancreatic carboxyl ester lipase are members of a gene family of neutral lipases [13–16]. Typical substrates for these enzymes are long-chain TAG which are separated from the aqueous medium by the surface phase. Thus, for catalysis some of these enzymes require the presence of cofactors. Lipoprotein lipase and pancreatic lipase utilize small protein cofactors, apolipoprotein C-II and co-lipase that influence on lipase stability and catalytic activity. Pancreatic CEL, also called bile-salt-stimulated lipase, is a key enzyme of dietary TAG absorption, hydrolyzing triacylglycerols to 2-monoacylglycerols and fatty acids. The enzyme has been documented to exhibit a number of carboxyl ester hydrolase activities including those of carboxylesterase, arylesterase, triacylglycerol lipase, lysophospholipase, cholesterol esterase and phospholipase A_1 [16]. Such a wide substrate specifity of CEL signifies that this enzyme might have pleiotropic biological functions. CEL has been reported in human plasma where it has the capability to modify normal human LDL and HDL structure and composition [9,11]. Lipoprotein lipase is the extrahepatic enzyme responsible for the hydrolysis of triglyceride-rich plasma lipoproteins, i.e. chylomicrons and very low density lipoproteins (VLDL) and has been hypothesized to exert either a pro- or an anti-atherogenic effect, depending upon its localization [17,18]. Although plasma LpL activity tends to drive lipoprotein metabolism in a nonatherogenic direction, LpL produced in the vascular wall may act as a proatherogenic protein [19]. The enzyme is also produced by monocyte-derived macrophages and vascular smooth muscle cells (VSMC), two prominent cellular components of atherosclerotic lesions [20,21]. An increasing amounts of evidence indicates that LpL also functions as a ligand associating with lipoproteins and promoting their binding to LDL receptor and proteoglycans. LpL possesses domains that bind both apo-B containing lipoproteins and proteoglycans, permiting this bridging action [17,20]. LpL has

been shown to mediate uptake of lipoprotein particles by vascular cells, to promote lipoprotein retention to the extracellular matrix, to induce the expression of the proatherogenic cytokine, tumor necrosis factor-α, and to enhance monocyte adhesion to endothelial cells [21].

Hepatic lipase catalyzes hydrolysis of TAG and the phospholipids of intermediate density lipoprotein (IDL) remnants, large LDL and high density lipoprotein (HDL) [13]. The primary role of HL is to maintain intracellular lipid homeostasis but its effects on different lipoprotein classes show that, potentially HL may promote as well as decrease atherogenesis. HL activity seems to correlate positively with atherosclerosis in hypertriglyceridemia and inversely in familial hypercholesterolemia [14]. In addition, HL serves as a ligand promoting cellular uptake of apolipoprotein, apo-B, containing remnant lipoproteins and HDL [22].

Recently, an endothelial lipase (EL) was discovered [15,23,24]. EL is synthetized by macrophages, and its endothelial expression suggest that EL may have a distinctive role in lipoprotein metabolism and vascular biology. In contrast to other lipases, EL has substantial phospholipase activity and lipoprotein phospholipids are its major substrate [24]. Thus, this enzyme provides the arterial intima with another regulated lipolytic mechanism for local release of fatty acids and lysophospholipids from lipoproteins that may influence atherogenesis [23].

4. Phospholipases

Phospholipase A_2s (PLA_2, phosphatide *sn*-2-acylhydrolases, EC 3.1.1.4) represent a superfamily of esterases that hydrolyze the *sn*-2 ester bond in phospholipids, releasing free fatty acids and lysophospholipids [25]. The ubiquitous nature of PLA_2s highlights the important role they play in many biological processes, including generation of pro-inflammatory lipid mediators such as prostaglandins and leukotriens, and regulation of lipid metabolism [26,27]. The PLA_2 family consists of two main kinds of enzymes: the secretory and the intracellular ones. A classification system is based on whether the PLA_2 is secreted from the cell ($sPLA_2$), is Ca^{2+}-dependent and cytosolic ($cPLA_2$) or Ca^{2+}-independent ($iPLA_2$) [28,29]. $sPLA_2$ activity is prominent in pathological processes, as the enzymes responsible for this activity are secreted by inflammatory cells upon their activation and by damaged tissues in inflammation-related diseases [29,30]. There exist groups IB, IIA, IIE, V and X secretory PLA_2s. Until now, only PLA_2-IB and PLA_2-IIA have been implicated in human disease. The catalytic activity of PLA_2 and the concentration of PLA_2-IB and PLA_2-IIA in serum are relatively low in healthy individuals. The findings that the serum content of PLA_2 correlates with the concentration of C-reactive protein (CRP) strongly support the view that PLA_2-IIA is an acute phase protein [31]. On the other hand, clinical studies indicate that an elevated plasma level of PLA_2 is a strong independent risk factor for coronary heart disease [32]. In addition, $sPLA_2$-IIA has been found in atherosclerotic plaques, where it is associated with both smooth muscle cells and macrophages [33].

Increased serum levels of PLA_2 were measured in patients with infectious and inflammatory diseases [27,34]. The involvement of PLA_2s in inflammation is the result of their ability to mobilize arachidonic acid from phospholipids, which serves as a substrate in the production of prostaglandins. Arachidonic acid is also the precursor of leukotriens which are formed via the lipoxygenase pathway. Eicosanoids are involved in a wide range of physiological activities, and their excessive production is involved in pathophysiology of numerous diseases, including inflammation, allergy, cancer development and cardiovascular disorders [29,30]. Other biological activities of PLA_2 include superoxide generation and lysosomal enzyme release [26]. In addition, lipolysis of LDL phospholipids by PLA_2 and lipid peroxidation generate lysoPC, which has potentially a multitude of pro-inflammatory effects in the arterial intima [10]. LysoPC and oxidized LDL can be chemoattractants for monocytes and T lymphocytes. They induce expression of growth factors and adhesion molecules in endothelial cells, and can be mitogenic for macrophages and smooth muscle cells [1,10]. Thus, PLA_2s have an important role in cellular injury via their ability to mediate inflammatory responses. In this case, inhibitors of the enzymes prove useful in determining biological roles of phospholipases A_2 in complex cellular processes and may also have therapeutic potential.

5. Lipase and phospholipase inhibitors

Studies on lipase inhibitors represent a critical area of investigation due to the potential pharmacological benefit of these compounds in treatment of inflammation and cell injury, and as a tool to investigate the role of lipases in physiological functions. Chemical inhibitors of lipases play an important role in elucidating their actions. There are many synthetic and natural compounds that inhibit enzymes containing Ser residue in their active site. Most of these inhibitors are amphoteric substances. Only a few substances directly interact with lipases themselves, one example being lipstatin, from *Streptomyces toxytricini*, which strongly inhibits lipases [35,36]. Tetrahydrolipstatin (THT; Orlistat) was identified as a nontoxic, active site inhibitor of mammalian lipases, including LpL, which could be used to effectively decrease lipolytic activity in plasma [37]. An inhibitory effect was also demonstrated of bis-2-oxo amide triacylglycerol analogues on human pancreatic lipase and human gastric lipase [38]. Lipoprotein lipase was also inhibited by alkanesulphonyl fluorides [39] but activated by a selective type III phosphodiesterase inhibitor, cilostazol [40].

Obliterative arteriosclerosis is a chronic disease and recent research suggests that inflammatory phenomena are the major determinants of its progression [2,3]. Beneficial effects of administering prostanoids, PGI_2 and PGE_2, have been reported in patients with advanced obliterative arteriosclerosis [41,42]. In vitro studies have shown that serum lipase (carboxyl ester lipase) activity was inhibited by prostanoids in different ways [43,44]. However, in men with atherosclerosis activity of this enzyme was stabilized by administration of the prostacycline analoque-*iloprost* and *prostavasin* (PGE_1). It is worth knowing that also the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (statins), i.e. simvastatin, used in hypercholesterolemia treatment affect the serum CEL activity [45].

The central role of PLA_2 in inflammation makes the enzyme a potential target for drug development. Theoretically, inhibition of PLA_2 may block formation of a wide variety of secondary inflammatory mediators. There is considerable interest in PLA_2 inhibitors as such components should have anti-inflammatory properties [46]. However, the major problem in the development of effective PLA_2 inhibitors involves the fact that cells contain different forms of PLA_2 that participate in normal phospholipid metabolism [25,27]. Therefore, when PLA_2 inhibition is considered for therapeutic use, the question of which PLA_2 plays the major role in the generation of pro-inflammatory lipid mediators is central. A number of PLA_2 inhibitors have been proposed as potential drugs for the treatment of inflammatory conditions [46,47]. These include cell-permeable inhibitors that might interfere with the vital phospholipid metabolism, carried out by the intracellular PLA_2s, and impair the cell viability [28]. The extracellulars PLA_2 inhibitors have been found effective in protecting membranes from different types of $sPLA_2$ inhibiting the activation of endogenous $sPLA_2$ and protecting cells from phospholipids hydrolysis. Extracellular PLA_2 inhibitors that fulfill these requirements have been designed and synthesized by linking known and novel PLA_2-inhibiting molecules, such as N-derivatised phosphatidyl-ethanolamine, to polymeric carriers [48]. These include natural macromolecules, such as hyaluronic acid, heparin and chondroitin sulphates, or polymers used in drug administration and clinical treatment (for example, hydroxy-ethylstarch, polygeline, carboxy-methylcelulose or dextrans). Due to their structure, the extracellular PLA_2 inhibitors have a dual effect in protecting the cell membrane from pro-inflammatory agents. The lipid moiety, which becomes incorporated into the cell membrane, suppresses activation of endogenous $sPLA_2$ and the polymeric carrier protects membranes from oxidation and other inflammatory stimuli [48]. Many of the early inhibitors of PLA_2 (e.g. dibucaine, mepacrine) were neither isoform-specific nor potent. Recently, two active site-directed inhibitors have been reported, arachidonyl trifluoromethyl ketone ($AACOF_3$), which displays specificity for the group IV $cPLA_2$ versus the group II $sPLA_2$ and a bromoenol lactone (BEL), which displays specificity for the myocardial $iPLA_2$ versus both groups I and III $sPLA_2$ [49]. Another fatty acid analog 20:4-PO(OMe)F (also known as MAFP) has been shown to irreversibly inactivate $cPLA_2$ and $iPLA_2$, possibly by phosphorylation of serine [50]. Phospholipid analogues that have a phosphonate or phosphate in place of the ester at the *sn*-2 position of phospholipids are tight-binding inhibitors of $sPLA_2$ but not of $cPLA_2$ [46].

In addition, a novel series of lipidic diamine and aminoalcohol derivatives with a PLA_2 inhibitor profile, showing potency and selectivity towards cytosolic and secretory PLA_2, has been developed [51]. Recently, a series of 1-(amidolinked)-alkyl)-pyrimidones **1** and a related series of 1-(arylpiperazinyl-amidoalkyl)-pyrimidones **2** have been described by Boyd et al. as inhibitors of PLA_2 type associated with lipoproteins [52]. 1-Me-pyrazol-4-yl and 5-(2-MeO-pyrimidin-5-yl) are good candidates to be used in evaluation of PLA_2 role in atherosclerosis. The promising drug candidate, LY315920/S-5920 [3-(aminooxoacetyl)-2-ethyl -1 - (phenylmethyl) - 1H-indol-4-yl-oxy]acetate, is currently undergoing phase II clinical evaluation [53]. Until now, however, no drug based on inhibition of PLA_2 has penetrated to clinical used. Thus, the development of highly specific lipolytic enzyme inhibitors will be of great interest, not only for studying their kinetic effects but also for potential pharmacological application.

6. A look toward the future

The focus of current therapy for atherosclerosis is the regulation of plasma lipid levels, particularly regulation of LDL cholesterol. Although the statins have achieved both medical and commercial success in this role, they are only partially effective. With the aim of treating many more members of the risk-exposed population the attention has been focused on targeting the inflammatory nature of this disease [2,3] and, in particular, the lipoprotein-associated phospholipase A_2 (PLA_2). Since various phospholipid derivatives and different PLA_2 enzymes seem to be involved in pathophysiology of different diseases, it remains unclear which lipid mediator, or which specific PLA_2 enzyme(s) should be inhibited for disease control [25]. Blocking a selective pathway of eicosanoids production diverts the arachidonate pool to the other pathway, not only failing to help but potentially even exacerbating the pathological conditions. When studying the effect of PLA_2 inhibition on cellular injury and death, careful selection of multiple inhibitors is of key importance, with special attention paid to experiments verifying that, in fact, PLA_2 is being inhibited. Clinical trials conducted thus far with PLA_2 inhibitors were relatively short and insufficient to allow chronic use of these drugs. Furthermore, recent evidence indicates that inhibitors can also exhibit marked side effects. These limitation in selective inhibition of lipolytic enzymes provide an explanation for the very limited success of this approach, despite the extensive effort invested in developing drugs that could suppress atherosclerosis.

The role of PLA_2 and other lipolytic enzymes in atherogenesis require more attention in future studies. In conclusion, there are numerous contributing factors that need to be studied and understood before inhibitor therapy becomes an option for the treatment for cardiovascular diseases.

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