Phospholipase A₂ in Vascular Disease

Eva Hurt-Camejo, Germán Camejo, Helena Peilot, Katarina Öörni, Petri Kovanen

Abstract—Secretory phospholipase A₂ (PLA₂) can be proatherogenic both in the circulation and in the arterial wall. In blood plasma, PLA₂ can modify the circulating lipoproteins and so induce formation of small dense LDL particles, which are associated with increased risk for cardiovascular disease. In the arterial wall, PLA₂ can hydrolyze lipoproteins. The PLA₂-modified lipoproteins bind tightly to extracellular proteoglycans, which may lead to their enhanced retention in the arterial wall. The modified lipoproteins may also aggregate and fuse, which can lead to accumulation of their lipids within the extracellular matrix. The PLA₂-modified particles are more susceptible to further modifications by other enzymes and agents and can be taken up by macrophages, leading to accumulation of intracellular lipids. In addition, lysophospholipids and free fatty acids, the hydrolysis products of PLA₂, promote atherogenesis. Thus, these lipid mediators can be carried, either by the PLA₂-modified lipoproteins themselves or by albumin, into the arterial cells, which then undergo functional alterations. This may, in turn, lead to specific changes in the extracellular matrix, which increase the retention and accumulation of lipoproteins within the matrix. In the present article, we discuss the possible actions of PLA₂ enzymes, especially PLA₂-IIA, in the arterial wall during atherogenesis. (Circ Res. 2001;89:298-304.)

Key Words: atherosclerosis ■ inflammation ■ cytokines ■ lipases ■ lysophospholipids

Phospholipase A₂ (PLA₂) is an enzyme that hydrolyzes the sn-2 ester bond in the glycerolipid phospholipids present in lipoproteins and cell membranes, forming nonesterified fatty acids (NEFAs) and lysophospholipids. These products may act either as intracellular second messengers or be further metabolized into mediators of a broad range of cellular processes. This review is motivated by evidence suggesting that the PLA₂ activity present in arterial intima-media and in plasma may be involved in atherogenesis. In vivo and in vitro results indicate that, in the arterial wall, PLA₂ may hydrolyze the phospholipids of the apolipoprotein (apo) B-100-containing lipoproteins retained in the arterial intima. The products of this hydrolysis, NEFAs and lysophospholipids, can trigger a variety of proinflammatory actions that lead to atherosclerotic plaque development. Furthermore, PLA₂-modified apoB-100 lipoproteins, which are more susceptible to further enzymatic and nonenzymatic modifications, induce accumulation of intracellular lipids and bind strongly to extracellular matrix proteoglycans. In addition, PLA₂ can induce aggregation and fusion of the matrix-bound lipoproteins and so further increase their binding strength to the matrix proteoglycans. Thus, in atherosclerosis-prone regions of arteries, PLA₂ may contribute to both intra- and extracellular accumulation of apoB-100 lipoproteins. On the other hand, circulating PLA₂ activity can modify LDL and HDL in plasma and may contribute to the generation of an atherogenic lipoprotein profile. Recent clinical studies indicate that an elevated plasma level of PLA₂ is a strong independent risk factor for coronary heart disease. Whether this is associated with...
the proatherogenic mechanisms of PLA₂ activity in plasma or with their actions in the arterial wall remains to be elucidated. The hypothesis of the potential involvement of sPLA₂-IIA in the pathogenesis of atherosclerosis is reinforced by in vivo data showing that transgenic mice expressing human sPLA₂-IIA have increased susceptibility to atherosclerosis.14,26 The PLA₂-induced changes in the physicochemical properties of circulating lipoproteins, mainly LDL, and their implications for atherogenesis were reviewed recently.31 In the present review, we discuss new evidence for potential atherogenic mechanisms of PLA₂ activity in the arterial wall.

The Family of PLA₂

The PLA₂ family comprises a rapidly growing group of intracellular and secreted enzymes. The mammalian secretory PLA₂ (sPLA) isoforms comprise the groups named IB, IIA, IIC, IID, IIE, IIF, V, X, and XII, along with a novel member, the type III sPLA recently cloned from human liver.32,33 Interestingly, human groups IIA, IIC, IID, IIE, IIF, and group V genes are clustered on chromosome 1, whereas the structurally more distant IB, X, XII, and III lie on different chromosomes.33 All sPLAs, except type III, have in common a low molecular weight, 13 to 16 kDa, several disulfide bridges, a Ca²⁺-dependent catalytic mechanism, and a well-conserved overall three-dimensional structure.32,34,35 Mammalian sPLA₂ appears to be implicated in a variety of physiological and pathological processes. These include lipid digestion, release of potent lipid mediators in response to cytokines stimulus,36 cell proliferation,37 control of virus and bacterial infection,38,39 removal of apoptotic or injured cells,40,41 phospholipid repair, lipoprotein catabolism,25,27,42,43 tumorigenesis, and inflammation (for reviews, see Uhl et al).44

sPLA₂-IIA in the Arterial Wall

sPLA₂-IIA was first isolated and purified from rheumatoid synovial fluid and is often referred to in the literature as synovial fluid sPLA₂. The enzyme was cloned in 1989, and the crystal structure at 2.2 Å was established in 1991.45,46 sPLA₂-IIA contains seven disulfide bridges, which confer a rigid structure and resistance to pH and to thermal and proteolytic denaturation. The protein has 23 cationic amino acid residues, arginine (Arg) and lysine (Lys), which contribute to its high positive charge (pI = 10.5). Specific cationic residues on its surface, among them Arg-7, Lys-10, and Lys-16, contribute significantly to the interfacial adsorption of the enzyme to the surface phospholipid monolayers of cell membranes, lipoproteins, and aggregated phospholipids.47 Furthermore, the positively charged regions may facilitate interaction with the negatively charged sulfated glycosaminoglycans of proteoglycans on the cell membranes and the extracellular matrix. These interactions with matrix and cell-surface proteoglycans help to localize the enzyme at such sites and to modulate its activity.23,48,49

In nonatherosclerotic human arteries, sPLA₂-IIA is mainly associated with the smooth muscle cells of the media, whereas in atherosclerotic plaques the enzyme is also found in macrophage-rich regions, in the acellular lipid core of atheromas, and in the extracellular matrix of the diseased intima in association with collagen fibers.15,50 Immunohistochemical studies performed by several research groups show that sPLA₂-IIA is found at all stages of atherosclerotic lesion development.15,50–55 Cytoplasmic PLA₂ (group IV) is also present in atherosclerotic lesions containing macrophages. However, the activity of sPLA₂-IIA is more prominent than that of the cytoplasmic enzyme in the same human plaque.52 In summary, immunohistochemical studies show that sPLA₂-IIA is present in normal arteries, and that, in early and late atherosclerotic lesions, its extracellular distribution and level of cell expression is increased, suggesting that the enzyme is implicated in atherogenesis.15,52,53

Most studies on sPLA₂-IIA expression in vascular cells have been performed with rat smooth muscle cells.56 Although some of these results can be extrapolated to human arterial smooth muscle cells, recent studies suggest that regulation of sPLA₂-IIA gene expression is cell- and species-specific.57,58 We reported recently that expression of both sPLA₂-IIA mRNA and protein by human arterial smooth muscle cells from the aorta and the coronary and uterine arteries requires conditions that promote cell differentiation in vitro.58 These in vitro results agree with immunohistochemical data showing that, in arteries, the main source of sPLA₂-IIA is smooth muscle cells.55,56 Electron microscopy shows that sPLA₂-IIA is stored intracellularly inside vesicles close to the smooth muscle cell membrane.50 Cytokines, in vitro, differentially modulate cell secretion and mRNA levels of sPLA₂-IIA. Thus, interferon-γ (IFN-γ) increases the expression of mRNA and sPLA₂-IIA protein secretion 2- to 6-fold, and, after addition of IFN-γ, this effect lasts up to 48 hours. On the other hand, tumor necrosis factor-α (TNF-α) stimulates sPLA₂-IIA secretion for only 4 hours, without detectable changes in mRNA levels. Interestingly, a similar effect of TNF-α is seen in sPLA₂-IIA transgenic mice.39,59 Interleukin-10 (IL-10), an anti-inflammatory cytokine, down-regulates IFN-γ, but not TNF-α, induction of sPLA₂-IIA secretion. In contrast to what was reported with rat smooth muscle cells, interleukin-1β (IL-1β) is not a strong inducer of sPLA₂-IIA in human arterial smooth muscle cells. Colocal-
ization of sPLA₂-IIA with the mRNA transcript for IFN-γ, IL-1β, and TNF-α in human atherosclerotic lesions supports a possible in vivo involvement of these cytokines in the regulation of sPLA₂-IIA gene expression and protein secretion in atherosclerotic plaques.54

Cytokines acting on tissues may also indirectly regulate the circulating levels of sPLA₂-IIA in plasma. sPLA₂-IIA is an acute-phase reactant and, in diseases that involve systemic inflammation such as sepsis, rheumatoid arthritis,60 and cardiovascular disease,30,61 its plasma levels are increased. Hepatocytes synthesize and secrete this enzyme in response to cytokines such as IL-6, TNF-α, and IL-1β,62 but not IFN-γ.58 In addition, peritoneal injections of IL-6, TNF-α, and IL-1β increase plasma levels of sPLA₂-IIA in transgenic mice expressing the gene of human sPLA₂-IIA.39,59 Together, these data suggest that hepatocytes and arterial smooth muscle cells may contribute to the bulk of circulating sPLA₂-IIA in plasma modulated by systemic inflammatory conditions. This hypothesis is illustrated in Figure 1.

The presence of active sPLA₂-IIA in plasma suggests that it may hydrolyze the surface phospholipid monolayer of plasma lipoproteins. A high plasma level of sPLA₂-IIA correlates positively with C-reactive protein (CRP) levels and predicts coronary events due to atherosclerosis28,30,60,61.63 This association is independent of other established risk factors.28 Furthermore, positive correlations between sPLA₂-IIA and soluble adhesion molecules, CRP, and antibody titers to IL-1β in plasma modulated by systemic inflammatory conditions. This hypothesis is illustrated in Figure 1.

Possible Atherogenic Actions of sPLA₂-IIA
The basic tenet of the response-to-retention hypothesis of atherosclerosis is that the lesions are the response of the arterial tissue to local accumulation and modification of apoB-100-containing lipoproteins in the intima of the arterial wall.69–71 Specific interactions of apoB-100-containing lipoproteins with chondroitin-sulfate proteoglycans such as versican or decorin appear to be important mechanisms contributing to their retention and modification.70 This early phenomenon is documented in ultrastructural studies showing accumulation of aggregated and fused lipoprotein particles in the subendothelial extracellular matrix before macrophages appear.72 Furthermore, early lesions in human aortas from fetuses of hypercholesterolemic women frequently contain LDL and oxidized LDL in the absence of monocyte-macrophages.73 Together, these observations suggest that modification of the lipoproteins retained in the extracellular matrix may precede lesion formation. In the arterial intima, there are proteolytic and lipolytic enzymes and pro-oxidant conditions capable of modifying the LDL retained in the extracellular matrix.15,74–78 Interestingly, the binding strength of LDL particles to extracellular matrix proteoglycans is increased by proteolytic and lipolytic modifications, whereas the binding strength of oxidized LDL is decreased.79 Indirect evidence indicates that once apoB-100 lipoproteins are in the intima, hydrolysis of phosphatidylcholine by PLA₂-like activity takes place rapidly.10–12 In addition, the concentration of lysophosphatidylcholine in rabbit atherosclerotic aorta is reported to be higher than in control tissue.13 Furthermore, active sPLA₂-IIA isolated from human arterial tissue is able to hydrolyze the phospholipids in LDL.15

In the arterial wall, sPLA₂-IIA may exert proatherogenic effects by the three main mechanisms illustrated in Figure 2. First, it may induce release of relatively high concentrations of lipid mediators such as NEFAs, oxidized NEFAs, and lysophosphatidylcholine (lyso-PC) bind albumin or remain associated with modified lipoproteins. This can induce aggregation and fusion of the lipoproteins, processes that are enhanced by intima proteoglycans. sPLA₂-IIA-modified LDL can be further modified by sphingomyelinase (SMase) and 15-lipoxygenase (15-LO). NEFAs, oxidized NEFAs, and lyso-PC may induce proinflammatory cellular processes, such as expression of adhesion molecules by endothelial cells, monocyte migration, and differentiation into macrophages, and so increase secretion of proteoglycans by smooth muscle cells (SMCs). Local release of cytokines can stimulate the synthesis and secretion of sPLA₂-IIA.
aggregation and fusion of the proteoglycan-bound apoB-100 lipoproteins.\textsuperscript{31,79,80} Treatment of LDL with sPLA\textsubscript{2}-IIA in the presence of physiological albumin concentration leads to the formation of small dense LDL particles with increased affinity for glycosaminoglycans and proteoglycans.\textsuperscript{23} As discussed above, this may take place in the plasma\textsuperscript{31} or in the arterial wall, where extracellular sPLA\textsubscript{2} can hydrolyze the phospholipids on the lipoprotein particles. Because PLA\textsubscript{2}-treated LDL particles have an increased affinity for extracellular proteoglycans, their residence time in the arterial wall is likely to increase. This provides the possibility for further modifications of these particles. In fact, PLA\textsubscript{2}-treated LDL particles are more susceptible to lipid peroxidation,\textsuperscript{66} generation of bioactive phospholipids,\textsuperscript{26} and hydrolysis by secretory sphingomyelinase.\textsuperscript{81} In addition, treatment of LDL with PLA\textsubscript{2} facilitates sphingomyelinase-induced aggregation and fusion of LDL particles.\textsuperscript{82} PLA\textsubscript{2} can also directly induce aggregation and fusion of LDL particles: treatment of proteoglycan-bound LDL with sPLA\textsubscript{2}-IIA leads to aggregation and subsequent fusion of the modified LDL particles.\textsuperscript{24} Interestingly, PLA\textsubscript{2} induces fusion of LDL particles only if the particles are bound to glycosaminoglycans either before, during, or after lipolysis.\textsuperscript{83} The interaction between LDL and glycosaminoglycans can apparently overcome the rigidifying effect of PLA\textsubscript{2}-induced hydrolysis on LDL particles.\textsuperscript{79} Thus, it can be hypothesized that if the small dense LDL particles generated by the action of sPLA\textsubscript{2}-IIA either in plasma or in the arterial intima bind to arterial proteoglycans, this interaction will then trigger aggregation and fusion of the bound LDL particles. Because each aggregate or fused particle contains several copies of apoB-100, it is not surprising that the aggregated/fused particles bind to proteoglycans even more tightly than do the small dense PLA\textsubscript{2}-treated LDL.\textsuperscript{82} Moreover, as the modified LDL particles aggregate or fuse, the capacity of arterial proteoglycans to bind LDL increases.\textsuperscript{24} Thus, aggregation and fusion of LDL particles in the arterial intima are likely to lead to accumulation of LDL-derived lipid particles within the extracellular matrix. Such progressive deposition of lipid within the extracellular matrix of the arterial intima is a central feature of atherogenesis and thus makes the arterial sPLA\textsubscript{2}-IIA a strong candidate as one of the key enzymes acting in the extracellular space during the development of atherosclerotic lesions. These proatherogenic mechanisms may be potentiated by the colocalization of sPLA\textsubscript{2}-IIA and apoB-100 lipoproteins with extracellular matrix proteoglycans in the intima. It should be stressed that the total hydrolysis of the phosphoglycerides from one LDL particle may generate more than 500 molecules of lysophospholipids and NEFAs. Thus, at sites of retention of apoB-100 lipoproteins in the arterial intima, NEFAs and lysophospholipids may reach high local concentrations. These bioactive products can induce different proatherogenic cellular processes and cell membrane perturbation. Human arterial smooth muscle cells exposed to albumin-bound NEFAs upregulate the synthesis of matrix proteoglycans.\textsuperscript{19} This results in a matrix that has a higher affinity for LDL, suggesting the possibility of a noxious cycle in which sPLA\textsubscript{2}-IIA products from apoB-100 lipoproteins entrapped in the intima trigger matrix changes, which, in turn, lead to further accumulation of lipoproteins. Peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)) agonists oppose this action of NEFAs, suggesting that the improved metabolism of NEFAs downregulates the increase in matrix synthesis in vivo.

**Molecular Basis and Effects of the sPLA\textsubscript{2}-IIA Interaction With Arterial Proteoglycans**

Extracellular sPLA\textsubscript{2}-IIA is associated with collagen fibers and proteoglycans in atherosclerotic lesions from human coronary arteries.\textsuperscript{50} The enzyme binds to collagen via a specific interaction with decorin, a proteoglycan containing the chondroitin and dermatan sulfate chains of the serine- and leucine-rich protein (SLRP) family that is associated with collagen fibers.\textsuperscript{84} In physiological salt and pH conditions, the enzyme is able to interact with both the glycosaminoglycan moiety and the core protein of decorin. In addition, sPLA\textsubscript{2}-IIA binds to the major proteoglycans of the arterial intima, versican and biglycan. However, with these molecules, the interaction takes place only through the glycosaminoglycan moiety. These associations with versican, biglycan, and decorin increase the hydrolytic activity of sPLA\textsubscript{2}-IIA toward the phospholipids on LDL or in micelles.\textsuperscript{23,48} Together with the remarkable stability of the enzyme, these results suggest that sPLA\textsubscript{2}-IIA could remain sequestered in an active form for prolonged periods in the extracellular environment of the arterial intima. This may be important for the normal functioning of the enzyme, but when it is secreted in excess, may potentiate its proatherogenic actions.

**Other PLA\textsubscript{2} Enzymes of Interest in Atherosclerosis**

Other sPLA\textsubscript{2} enzymes may have relevance for the physiology and pathology of the arterial wall. Plasma platelet-activating factor (PAF) acetylhydrolase (PAF-AH or group VII) is a serine lipase that hydrolyzes the sn-2 ester bond of PAF and thus attenuates its bioactivity. Some experimental and clinical studies suggest that PAF acetylhydrolase has anti-inflammatory properties.\textsuperscript{85} In contrast, this enzyme was suggested to be a proinflammatory agent because of its capacity to hydrolyze oxidized phospholipids releasing lyso-phospholipids and oxidized fatty acids from free radical–oxidized lipoproteins retained in the arterial intima.\textsuperscript{86} PAF-AH circulating in plasma is associated with lipoproteins, mainly LDL, and, because of its PLA\textsubscript{2} property, it is also known as lipoprotein-associated PLA\textsubscript{2}.\textsuperscript{87} In addition, this enzyme is expressed by macrophages in human and rabbit atherosclerotic lesions.\textsuperscript{88} Recently, lipoprotein-associated PLA\textsubscript{2} was reported to be an independent risk factor for coronary heart diseases in hypercholesterolemic patients.\textsuperscript{29} Furthermore, inhibition of the enzyme slows down atherogenesis in LDL receptor–defective Watanabe rabbits.\textsuperscript{86} This suggests that lipoprotein-associated PLA\textsubscript{2} could become a new target for therapeutic antiatherosclerotic intervention.\textsuperscript{86}

There are no studies that could help us to conclude which of these two phospholipases, sPLA\textsubscript{2}-IIA or the lipoprotein-associated enzyme, has the greater potential for atherogenesis. However, compared with the lipoprotein-associated PLA\textsubscript{2}, sPLA2-IIA hydrolyzes intact as well as oxidized...
phospholipids, thus generating NEFAs, oxidized NEFAs, and lysophospholipids from a broader spectrum of substrates. In addition, its expression in the mouse increases the susceptibility to atherosclerosis and its plasma levels predict coronary events in patients with well-defined atherosclerosis. Therefore, sPLA₂-IIA may also be a potential target for therapeutic antiatherogenic agents. In vivo studies in models of atherosclerosis with specific inhibitors of each enzyme will be necessary to evaluate their relative relevance for the disease in vivo.

Although not yet directly implicated in arterial disease, the group V and group X sPLA₂ enzymes are expressed in macrophages and mast cells (group V) and in spleen and thymus (group X), and a novel sPLA₂, group XII, is found in stimulated type 2 helper T cells. Therefore, these enzymes may be prominent at sites where macrophages, lymphocytes, and mast cells accumulate in atherosclerotic lesions. Groups V and X are more efficient than sPLA₂-IIA in hydrolyzing phosphatidylcholine vesicles and the outer plasma membrane of intact mammalian cells. Thus, their capacity to release fatty acids and lysophospholipids, which in turn induce cellular eicosanoid production, may also be higher than that of sPLA₂-IIA. The presence of tryptophan and less basic residues in the interface-binding region of these enzymes appears to contribute to their efficient hydrolysis of cell-membrane phospholipids. However, the possible involvement of sPLA₂-V, sPLA₂-X, and sPLA₂-XII and their potential overlapping function with sPLA₂-IIA in sustaining a chronic inflammation in atherogenesis remains to be studied.

In addition to the secretory phospholipases mentioned above, an endothelial lipase (EL) was discovered recently. EL belongs to the family of triglyceride lipases. However, in contrast to other lipases, EL has substantial phospholipase activity and lipoprotein phospholipids are its major substrate. EL is synthesized by macrophages, and its expression by coronary artery endothelial cells is upregulated by inflammatory cytokines. 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.

**Future Directions**

For many years, researchers and the pharmaceutical industry have studied the possibility of using inhibitors of sPLA₂ to treat proinflammatory conditions. However, new knowledge about the existence of many types of sPLA₂ highlights the need for specific inhibitors. The recent elucidation of the human and other genomes may add new PLA₂ genes to this already growing family. This information, combined with the use of proteomics and genetically manipulated mouse models of atherosclerosis, should increase our knowledge of the specific and potentially overlapping roles of individual phospholipases as mediators of physiological and pathological processes. Hopefully, such understanding will enable the development of specific agents aimed at decreasing the potential contribution of individual secretory phospholipases to atherosclerotic disease.

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