Secreted phospholipase A₂, lipoprotein hydrolysis, and atherosclerosis: integration with lipidomics

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Abstract Phospholipase A₂ (PLA₂) is a group of enzymes that hydrolyze the sn-2 position of glycerophospholipids to yield fatty acids and lysophospholipids. Of many PLA₂s or related enzymes identified to date, secreted PLA₂s (sPLA₂s) comprise the largest family that contains 10 catalytically active isozymes. Besides arachidonic acid released from cellular membranes for eicosanoid synthesis, several if not all sPLA₂s have recently been implicated in hydrolysis of phospholipids in lipoprotein particles. The sPLA₂-processed low-density lipoprotein (LDL) particles contain a large amount of lysophospholipids and exhibit the property of “small-dense” or “modified” LDL, which facilitates foam cell formation from macrophages. Transgenic overexpression of these sPLA₂s leads to development of atherosclerosis in mice. More importantly, genetic deletion or pharmacological inhibition of particular sPLA₂s significantly attenuates atherosclerosis and aneurysm. In this article, we will give an overview of current understanding of the role of sPLA₂s in atherosclerosis, with recent lipidomics data showing the action of a subset of sPLA₂s on lipoprotein phospholipids.

Keywords Lipids · Enzymes · Mass spectrometry

Abbreviations
ESI-MS Electrospray ionization-mass spectrometry
HDL High-density lipoprotein
LDL Low-density lipoprotein
LPA Lysoosphatadtic acid
LPC Lysoosphatidylicoline
LPE Lysoosphatidylethanolamine
PC Phosphatidylcholine
PG Prostaglandin
PUFA Polyunsaturated fatty acid
SM Sphingomyelin
sPLA₂ Secreted phospholipase A₂

Classification of sPLA₂s

The sPLA₂ family represents structurally related, disulfide-rich, low molecular weight, lipolytic enzymes with a His-Asp catalytic dyad. sPLA₂s occur in a wide variety of vertebrate and invertebrate animals, plants, bacteria, and viruses, and 10 catalytically active sPLA₂ isoforms (IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XIIA) are identified in mammals. Of these, sPLA₂s belonging to the group I/II/V/X collection are closely related 14–19-kDa proteins of secreted enzymes with a highly conserved Ca²⁺-binding loop and a His-Asp catalytic site. In addition to these elements, there are six absolutely conserved disulfide bonds and up to two additional unique disulfide bonds, which contribute to the high degree of stability of these enzymes. Group III and group XII sPLA₂s share little homology with the I/II/V/X collection of sPLA₂s except for the Ca²⁺-binding loop and the catalytic site, thereby representing the distinct group collections. Unlike intracellular PLA₂s, sPLA₂s...
hydrolyze glycerophospholipids only in the presence of millimolar concentrations of Ca\(^{2+}\), suggesting that they primarily act on “extracellular” substrates. Since individual sPLA\(_2\)s display distinct cellular/tissue distributions and substrate head group/fatty acid specificities, they may play non-redundant, isoform-specific roles in vivo. The latest biochemistry and biology of the sPLA\(_2\) family have been detailed in recent reviews [1, 2].

**Lipoprotein hydrolysis by sPLA\(_2\): in vitro studies**

Lipoprotein modification and atherosclerosis; a general view

A lipoprotein is a biochemical assembly that contains both proteins and lipids whose function is to transport water-insoluble lipids in the water-based bloodstream. Examples include the high-density (HDL) and low-density (LDL) lipoproteins, which enable fats to be carried between the blood and tissues. Since higher levels of LDL particles promote health problems and cardiovascular disease typically known as atherosclerosis, they are often called the **bad cholesterol** particles, as opposed to HDL particles that are frequently referred to as **good cholesterol** or **healthy cholesterol** particles. LDL particles vary in size and density, and studies have shown that high plasma levels of small dense LDL particles rather than larger and less dense LDL particles well correlate with a higher risk for coronary heart disease. The surfaces of LDL and HDL are surrounded by phospholipids, mainly phosphatidylcholine (PC), which, as a matter of fact, serves as a very good “extracellular” target of several if not all sPLA\(_2\) isoforms.

It has been believed that a key step of pro-atherogenic small-dense LDL generation is oxidative modification of the polyunsaturated fatty acids (PUFAs) in phospholipids on LDL surface. However, the “oxidation hypothesis of atherosclerosis” [3] still remains inconclusive, as oxidation alone cannot fully explain the accumulation of large amounts of lipids and lysophosphatidylcholine (LPC) in foam cells and fatty streak lesion formation [4]. Current knowledge suggests that sPLA\(_2\)-mediated modification of lipoproteins plays a role in the development of atherosclerosis [5, 6]. This idea originally arose from the following key observations. Hydrolysis of PC in lipoproteins by sPLA\(_2\)s produces free fatty acids (typically unsaturated) and LPC, which can trigger vasoactive, chemotactic, and pro-inflammatory actions leading to the acceleration of atherosclerosis. Hydrolysis of LDL by sPLA\(_2\) correlates with production of the more atherogenic, small-dense, modified LDL with increased net negative charge, whereas hydrolysis of HDL reduces the capacity of this anti-atherogenic particle to promote cholesterol efflux from lipid-rich foam cells. Modified LDL retained in atherosclerotic lesions contains less PC and more LPC than does circulating LDL, suggesting that arterial LDL undergoes lipolytic modification by certain extracellular PLA\(_2\) enzyme(s) at lesion sites. Further, clinical analyses have shown that elevated plasma PLA\(_2\) activity (likely sPLA\(_2\)-IIA) is an independent risk factor for cardiovascular disease [7, 8], and a low content of surface phospholipids often characterizes the small-dense LDL and HDL subclasses [9].

Hydrolysis of lipoprotein-bound phospholipids by sPLA\(_2\)s can give rise to the two pro-atherogenic and pro-inflammatory lipid products, lysophospholipids and fatty acids. LPC modulates the expression of a number of proteins such as cytokines, chemokines, growth factors, adhesion molecules, inducible nitric oxide synthase and cyclooxygenase-2 [10]. LPC plays an ethiologic role in atherosclerosis, is a major constituent of atherogenic lipoproteins [11], and exhibits pro-inflammatory functions including activation of macrophages as well as induction of chemotactic factors and adhesion molecules in endothelial cells [12]. Lysophosphatic acid (LPA), an autotaxin-hydrolyzed product of LPC that elicits numerous effects on cells of the cardiovascular system, induces the formation of arterial neointima lesions, a prelude of atherosclerosis, through the PPAR\(\gamma\)-dependent mechanism [13]. LPA accumulates in the lipid-rich core of human carotid atherosclerotic plaques [14]. Arachidonate-oxygenated lipid mediators, including prostaglandins (PGs) and leukotrienes, also have diverse effects on atherosclerosis, as evidenced by studies employing knockout mice for their receptors or biosynthetic enzymes. For instance, gene ablation of thromboxane A\(_2\) receptor or PGE\(_2\) synthase ameliorates, whereas that of PG\(_{1}\) receptor or PGD\(_2\) synthase exacerbates, the experimental atherosclerosis in mice [15–17]. Mice lacking 5- or 12/15-lipoxygenase are also partially protected from the development of atherosclerosis [18, 19]. Thus, increased production of these pro-atherogenic lipid mediators may account, at least in part, for the pro-atherogenic action of sPLA\(_2\)s. A proposed idea for the mechanistic action of sPLA\(_2\)s on the development of atherosclerosis is illustrated in Fig. 1.

However, a series of initial studies describing the relationship among sPLA\(_2\), lipoprotein hydrolysis and atherosclerosis have some concerns that should be interpreted more carefully. First, many studies using sPLA\(_2\)s from snake or bee venom could be misleading, since the properties of venom sPLA\(_2\)s are distinct from those of mammalian sPLA\(_2\)s. Second, even if mammalian sPLA\(_2\)s were used, their concentrations employed were often very high (>100 nM) that could be out of the physiological level. Third, many investigators had an incorrect recognition that all or most mammalian sPLA\(_2\)s are induced during inflammation and can exist in the plasma. However, it is only sPLA\(_2\)-IIA that is strongly induced under pathologic...
In the past five years, several studies have examined the hydrolytic activity of human sPLA₂s toward LDL- or HDL-associated phospholipids using mass spectrometry (MS). These approaches have delineated the fundamental differences in lipoprotein hydrolysis by distinct human sPLA₂s. Several quantitative analyses have shown that sPLA₂-V and -X are 20 ~30 times more reactive on PC in HDL and LDL than sPLA₂-IB and -IIA [21–23]. Interestingly, sPLA₂-X hydrolyzes arachidonate- and linoleate-containing PC species preferentially, group V hydrolyzes oleoyl- and linoleate-PC in preference to arachidonate-PC, and sPLA₂-IIA hydrolyzes randomly all diacyl molecular species. The hydrolysis of minor phospholipid species (e.g., phosphatidylinositol and phosphatidylserine) in HDL and LDL by sPLA₂-V and -X is low relative to that of PC [24]. As a result, these acidic phospholipids remain at higher levels in both LDL and HDL, thereby increasing the acidity of the modified particles. Although the activity of sPLA₂-IIA on lipoproteins is relatively weak (also see below), it can hydrolyze acute phase HDL 2–3-fold more efficiently than normal HDL, with preferential attack on PC with oxygenated PUFAs [25]. LDL hydrolysis by sPLA₂-V is also affected by the contents of other lipid components such as sphingomyelin (SM) and neutral lipids, since higher percentages of SM interfere with LDL hydrolysis by sPLA₂-V and -X [22] and since LDL from patients with type 2 diabetes is more susceptible than that from normal subjects to sPLA₂-V hydrolysis [26, 27].

We performed electrospray ionization MS (ESI-MS) to directly compare the hydrolytic activity of six human sPLA₂ isoforms, IIA, IIE, IIF, III, V and X, on PC in human LDL and HDL particles (for more details, please see [28]). Both LDL and HDL particles contained three major PC molecular species (C16:0–18:2, C18:0–18:2 and C18:0–20:4) and only trace levels of LPC molecular species.
(C16:0 and C18:0). When LDL was treated with a low concentration (10 nM) of sPLA$_2$S for 4 h, three sPLA$_2$S, namely III, V and X, robustly increased both LPC species (Fig. 2a). The capacities of these three enzymes to produce LPC and lysophosphatidylethanolamine (LPE) from LDL were nearly comparable (Fig. 2b). Marked increases of LPC species were also observed when HDL was incubated with 10 nM sPLA$_2$-V or -X, whereas the activity of sPLA$_2$-III on HDL-bound PC at this concentration was modest even though significant (Fig. 3a). Notably, all PC species were dramatically reduced by sPLA$_2$-X, linoleate-containing PC species were reduced in preference to arachidonate-containing PC by sPLA$_2$-V, and arachidonate-containing PC was preferentially reduced by sPLA$_2$-III, revealing differences in the potency and fatty acid selectivity of these three sPLA$_2$S on HDL-associated PC. In addition to LPC, LPE was also greatly increased when HDL was treated with sPLA$_2$-V or sPLA$_2$-X, and to a lesser extent with sPLA$_2$-III (Fig. 3b). The ability of sPLA$_2$-IIA and -IIE to hydrolyze PC in LDL and HDL was minimal even at 50 nM, while sPLA$_2$-IIIF at this concentration showed significant activity with hydrolysis of arachidonate-PC to produce C16:0-LPC in LDL and to produce both C16:0- and C18:0-LPC in HDL with hydrolysis of arachidonate-PC to produce C16:0-LPC was also greatly increased when HDL was treated with those of other sPLA$_2$s and it is the only sPLA$_2$ isoform species were reduced in preference to arachidonate-containing PC by sPLA$_2$-V, and arachidonate-containing PC was preferentially reduced by sPLA$_2$-III, revealing differences in the potency and fatty acid selectivity of these three sPLA$_2$S on HDL-associated PC. In addition to LPC, LPE was also greatly increased when HDL was treated with sPLA$_2$-V or sPLA$_2$-X, and to a lesser extent with sPLA$_2$-III (Fig. 3b). The ability of sPLA$_2$-IIA and -IIE to hydrolyze PC in LDL and HDL was minimal even at 50 nM, while sPLA$_2$-IIIF at this concentration showed significant activity with hydrolysis of arachidonate-PC to produce C16:0-LPC in LDL and to produce both C16:0- and C18:0-LPC in HDL (Fig. 4).

Taking these results altogether, the rank order of the hydrolytic potency of various human sPLA$_2$S, as evaluated by ESI-MS, is X > V > III > II > IIIF, IIIE for both LDL and HDL. This order appears to roughly correlate with their ability to interact with PC-rich vesicles and with PC-rich cellular plasma membranes [1, 2]. Note that, although sPLA$_2$-IIA did not show a detectable level of activity in our experimental setting (see above), previous studies employing very high concentrations of sPLA$_2$-IIA have shown that it could hydrolyze lipoprotein-bound PC to some extents, particularly oxidized lipoproteins [25, 29, 30]. Since the expression level of sPLA$_2$-IIA is considerably higher than those of other sPLA$_2$s and it is the only sPLA$_2$ isoform detected in the circulation of mammals (except mice) [1, 2], it is still plausible that sPLA$_2$-IIA participates in atherosclerotic lipoprotein hydrolysis in vivo, as discussed below.

**Cellular actions of sPLA$_2$-treated LDL**

Atherosclerosis, and the resulting coronary heart disease and cerebral stroke, represent one of the most common causes of death in industrial nations. Cholesterol-engorged macrophages and their detritus following cell death comprise a major volume of early fatty streak plaques as well as the most typical advanced lesions of arteries. Unregulated uptake of cholesterol by macrophages results in the accumulation of multiple lipid droplets leading to the aptly named “foam cell” phenotype [31]. Numerous studies have described a variety of foam cell responses that would contribute to the growth and rupture of the vessel wall plaques of atherosclerosis, and the cholesterol-loaded macrophages appear to contribute to the inception of the process, the lethal conclusion in plaque rupture, and the triggering of the occlusive thrombosis [32]. Oxidized LDL, a generally recognized form of modified LDL, is believed to bring about in the sub-endothelial space where circulating anti-oxidant defense are less effective. Mildly oxidized LDL can stimulate the release of chemokines by endothelial cells, increase the adherence and penetration of monocytes, and induce scavenger receptor A (SR-A) and CD36 expression in macrophages [33–35]. Extensively oxidized LDL becomes a ligand for SR-A and other scavenger receptors that contribute to foam cell formation by facilitating uptake of lipoprotein particles.

The sPLA$_2$-hydrolyzed LDL particles, with increased LPC contents and small diameter, can potently promote lipid droplet accumulation in macrophages, a process reminiscent of foam cell formation [28, 36, 37]. Indeed, as does oxidized LDL, sPLA$_2$-modified LDL shows some typical features of pro-atherogenic particles, such as increased affinity for matrix proteoglycans and propensity of aggregation [36, 38]. Association of sPLA$_2$-IIA or -V with matrix proteoglycans increases the hydrolysis of LDL-associated PC [39–41]. Furthermore, treatment with sPLA$_2$-IIA renders LDL more susceptible to oxidative modification and increases its affinity for matrix proteoglycans. Conceivably, the close spatial contact between sPLA$_2$-IIA and LDL on proteoglycans may allow their efficient interaction, and sPLA$_2$-IIA can promote aggregation and fusion of the proteoglycan-bound LDL, leading to progressive deposition of lipids within the extracellular matrices of the arterial intima, a central feature of atherosclerosis [39–41]. Uptake of sPLA$_2$-V-treated LDL by macrophages depends on binding to syndecan 4, a cellular proteoglycan, rather than to the scavenger receptors SR-A and CD36 [41, 42]. LDL lipolysis by sPLA$_2$-V results in production of free fatty acids such as oleic and linoleic acids, which augment TNFα and IL-6 secretion by macrophages [43]. Lipolytic modification of HDL by sPLA$_2$-V or -X reduces its capacity to promote cholesterol efflux from lipid-loaded macrophages, thereby reducing its anti-atherogenic function [44]. The sPLA$_2$-modified LDL can also affect the function of endothelial cells. The pan-sPLA$_2$
sPLA₂ and Atherosclerosis: in vivo studies

Expression of sPLA₂ in atherosclerotic plaques

sPLA₂-IIA is located in macrophage-rich regions, lipid cores of atheromas, and the extracellular matrix of the diseased intima in association with collagen fibers in human atherosclerotic lesions [51]. sPLA₂-V is also enriched in atherosclerotic lesions of humans and experimental animals, in which it is associated with smooth muscle cells and also surrounding foam cells in lipid core areas [52, 53]. A hyperlipidemic high-fat diet up-regulates sPLA₂-V expression in the aorta, and apoE⁻/⁻ x Ldlr⁻/⁻ mice, in which atherosclerosis develops spontaneously, show elevated aortic sPLA₂-V expression [52]. sPLA₂-X is also immunohistochemically detected in atherosclerotic lesions in both humans and apoE⁻/⁻ mice. In humans, sPLA₂-X is detected in the intima where it is localized in the majority of foam cells and in phenotypically de-differentiated smooth muscle cells resembling myofibroblasts as well as in the extracellular matrix, but not detectable in T-lymphocytes and in the lesion-free areas. sPLA₂-III is focally expressed in advanced lesions of atheroma in human and apoE⁻/⁻ mice, mainly in macrophages and smooth muscle cells [28]. Other sPLA₂s (IID, IIIE, IIIF) are also detected by immunohistochemistry and in situ hybridization in human atherosclerotic plaques, with sPLA₂-IIIF exhibiting the most notable induction in accordance with the developmental process of atherosclerosis [54].

sPLA₂-IIA

The principal experimental evidence for the potential role of mammalian sPLA₂ in atherosclerosis has arisen from studies employing transgenic mice overexpressing human sPLA₂-IIA (PLA2G2A-Tg) [55], beyond the fact that the C57BL/6 mouse strain intrinsically lacks sPLA₂-IIA as a result of a natural mutation of its gene [56]. PLA2G2A-Tg mice maintained on high-cholesterol atherogenic diet exhibit increased atherosclerotic lesions [55]. In these mice, sPLA₂-IIA is present in atherosclerotic lesions in the aorta, and the plasma level of HDL is lower and that of LDL is slightly higher in PLA2G2A-Tg mice than those in control mice. Of importance, transplantation of bone marrow cells from PLA2G2A-Tg mice into recipient Ldlr⁻/⁻ mice results in a significant increase in the extent of atherosclerosis in the aortic arch and sinus despite the absence of alteration in lipoprotein composition, suggesting that macrophage-derived sPLA₂-IIA can exert a local pro-atherogenic effect with enhancement of collagen deposition by a process independent of systemic lipoprotein metabolism [57]. Thus, even though the hydrolytic action of sPLA₂-IIA on PC in LDL and HDL is relatively low, it is still possible that only local modification of lipoproteins by this enzyme within vascular walls is sufficient for development of atherosclerosis.

sPLA₂-V

The findings that sPLA₂-V can hydrolyze LDL- and HDL-associated PC far more efficiently than does sPLA₂-IIA and that the LDL modified by sPLA₂-V efficiently induces macrophage foam cell formation, as described above, have led to the idea that this enzyme is more important than sPLA₂-IIA for the promotion of atherosclerosis [58]. Importantly, Ldlr⁻/⁻ mice subjected to retrovirus-mediated gene transfer of Pla2g5 cDNA have increased lesion area in the ascending aortic root with a concomitant elevation of regional collagen deposition, whereas mice transplanted with bone marrow cells from Pla2g5⁺/⁺ mice show reduced atherosclerosis in the aortic arch and thoracic aorta [53]. This result clearly indicates that sPLA₂-V exerts a pro-atherogenic function in vivo. Surprisingly, however, reduction of atherosclerotic lesion size is not evident in apoE⁻/⁻ mice reconstituted with Pla2g5⁻/⁻ bone marrow cells, probably because the lipoprotein lipid compositions are distinct.
between the Ldlr−/− and apoE−/− backgrounds [59]. Nevertheless, the collagen content of the plaques is significantly reduced in lesions of apoE−/− mice lacking sPLA2-V. It should be noted, however, that these bone marrow transplantation approaches could assess the role of sPLA2-V expressed only in macrophages or other hematopoietic cells. Hence, the impact of sPLA2-V expressed in non-hematopoietic cells on atherosclerosis still remains unknown. Of note, a recent tagging single nucleotide polymorphism analysis has demonstrated an association of the human PLA2G10, but not PLA2G2A, gene haplotype with the plasma levels of LDL and oxidized LDL in patients with type 2 diabetes [26].

sPLA2-X

sPLA2-X also potently hydrolyzes phospholipids in LDL and HDL in vitro, with an effect even superior to that of sPLA2-V (see above). A recent study has demonstrated that the deficiency of sPLA2-X on the ApoE−/− background significantly reduces the incidence and severity of angiotensin II-induced abdominal aortic aneurysm and atherosclerosis, accompanied by reduction of pro-inflammatory mediators [60]. Moreover, another study using Pla2g10−/− macrophages has provided an additional view that sPLA2-X negatively regulates cholesterol efflux from macrophages through altering the liver X receptor (LXR)-dependent expression of ABC transporters [61]. These results support the idea that sPLA2-X has a pro-atherogenic role in vivo. In humans, however, non-synonymous polymorphism in the PLA2G10 gene, which leads to a profound change in the expression and activity of sPLA2-X, has no detectable impact on cardiovascular disease risk, whereas another polymorphism located in the 5′-untranslated region is associated with a decreased, rather than increased, risk of recurrent cardiovascular events [62]. Considering that sPLA2-X can also exert an anti-inflammatory function probably through producing anti-inflammatory PUFAs or their metabolites [48], the mechanistic action of sPLA2-X in atherosclerosis could not be simply explained only by alterations in the lipoprotein modification. Further investigation should be needed to elucidate the role of sPLA2-X.

To assess whether sPLA2-X has the capacity to hydrolyze lipoprotein PC in vivo, we examined the lipoprotein profiles in plasma of transgenic mice overexpressing human sPLA2-X (PLA2G10-Tg) [63] in comparison with littermate wild-type (WT) mice. Plasma PL2A activity, as evaluated by release of [14C]linoleic acid from 1-palmitoyl-2-[14C]linoleoyl-phosphatidylethanolamine, was dramatically elevated in PLA2G10-Tg mice over WT mice (Fig. 5a). sPLA2-X is synthesized as an inactive pro-enzyme, and cleavage of the N-terminal propeptide gives rise to a mature active enzyme, which further undergoes N-glycosylation [64, 65]. Accordingly, sPLA2-X proteins (mature, pro- and glycosylated forms) were detected in the plasma of PLA2G10-Tg mice, as assessed by immunoblotting using anti- sPLA2-X antibody (Fig. 5b). Lipids were extracted from LDL and HDL of these mice and subjected to ESI-MS for phospholipid analysis (Fig. 5c–e). In both LDL and HDL, there were robust increases in C16:0- and C18:0-LPC (Fig. 5d and e), with a concomitant decrease in all PC molecular species (Fig. 5c), in PLA2G10-Tg mice relative to WT mice. These results suggest that sPLA2-X overexpressed in PLA2G10-Tg mice hydrolyzed LDL- and HDL-associated PC robustly in vivo.

It should be noted, however, that endogenous sPLA2-X was undetectable in the plasma of WT mice (Fig. 5b), and that we observed no difference in the lipoprotein composition between Pla2g10−/− and littermate Pla2g10+/+ mice under physiological conditions [66]. Therefore, even though the above study employing PLA2G10-Tg mice has pointed that sPLA2-X has the capacity to hydrolyze lipoprotein PC in vivo, its physiological importance remains unclear. Presumably, under certain pathological conditions, the expression level or proteolytic processing of sPLA2-X is increased locally (e.g. in atherosclerotic lesions), where it could contribute to hydrolysis of lipoprotein PC. Indeed, a study using PLA2G10-Tg mice has provided evidence that the proteolytic processing of sPLA2-X is facilitated at inflamed sites [63].

sPLA2-III

sPLA2-III can efficiently hydrolyze PC in LDL and to a lesser extent in HDL (see above). sPLA2-III-modified LDL, like sPLA2-V- or sPLA2-X-treated LDL, facilitates the formation of foam cells from macrophages ex vivo [28]. After intake of an atherogenic diet, aortic atherosclerotic lesions are more severe in mice with transgenic overexpression of human sPLA2-III (PLA2G3-Tg) than in control mice on an apoE−/− background [28]. In these mice, plasma LDL and HDL are significantly hydrolyzed by the enzyme, and peritoneal macrophages readily store lipid droplets in the cytoplasm after exposure to LDL ex vivo. Lipidomics studies demonstrate the elevation of LPC as well as thromboxane A2 and 12-hydroxyeicosatetraenoic acid, which are arachidonate-derived products by activated...
platelets, in plasma of PLA2G3-Tg mice relative to WT mice. Interestingly, PLA2G3-Tg mice also develop systemic inflammation [67], suggesting that the elevated inflammatory state in the vascular wall may have an additional impact on the promotion of atherosclerosis in these mice. Although these observations suggest a potential functional link between sPLA2-III and atherosclerosis, its pathological relevance awaits further study employing Pla2g3−/− mice.

Pharmacologic effect of sPLA2 inhibitor on atherosclerosis

Accumulating evidence as mentioned above suggests that sPLA2 may represent a novel target for atherosclerosis and associated cardiovascular diseases. The potent sPLA2 inhibitors, which broadly inhibits sPLA2s in the group I/II/V/X branch at low nM potency in vitro, include the
The development of these compounds involves structure-guided improvement of binding capacity starting with a lead compound obtained through high-throughput screening and making use of the X-ray structure of human sPLA2-IIA [69]. Interestingly, A-002 (1H-indole-3-glyoxamide or varespladib), a lead compound of this pan-sPLA2 inhibitor series, can decrease the area of atherosclerotic lesions dramatically, accompanied by a 1.4-fold increase in HDL, in apoeE−/− mice fed a high-fat diet [70–72]. Combinational treatment of animals with pravastatin (a member of the drug class of statins (HMG-CoA reductase inhibitors)) and A-002 decreases the lesion area and plasma cholesterol level even more, suggesting a synergistic effect between the two agents in amelioration of atherosclerosis through decreased levels of systemic inflammation or circulating lipids. A-002 treatment also stabilizes the plaque architecture. Because apoeE−/− mice (C57BL/6 background) intrinsically lack sPLA2-IIA due to a natural mutation [56], the anti-atherosclerotic effect of A-002 could be attributable to the inhibition of other sPLA2 isoforms, probably sPLA2-V or -X (note that A-002 does not inhibit sPLA2-III, an atypical sPLA2). Furthermore, a phase II double-blind, randomised, placebo-controlled trial to assess the effects of A-002 in human patients with coronary heart disease has demonstrated that the serum concentration of sPLA2 (most likely sPLA2-IIA), as well as the levels of vascular (oxidized LDL) and general (C-reactive protein) inflammation markers, decreases progressively to nearly an order of magnitude less than the baseline, with no increase in adverse events [73]. A-002 also reduces the concentration of LDL cholesterol and the number of LDL particles, mainly by reducing small-dense LDL. Thus, A-002 shows promising reduction of biomarkers and effects on surrogate endpoints, encouraging further investigation of whether it can reduce cardiovascular disease events without any other off-target toxicity. Although it is uncertain whether A-002 exerts its anti-atherosclerotic effect in humans by inhibiting circulating sPLA2-IIA, plaque-associated sPLA2-V and -X, or both, these animal and early-phase clinical studies nevertheless suggest that sPLA2s could be exciting therapeutic targets for atherosclerosis.

Concluding Remarks

In this article, we have highlighted a current view of the role of sPLA2s in lipoprotein hydrolysis and atherosclerosis. Needless to say, MS-based lipidomics has greatly contributed to expanding our understanding of sPLA2-mediated hydrolysis of lipoprotein phospholipids. sPLA2s have also been implicated in various biological processes, such as asthma, arthritis, cancer, antimicrobial defense and reproduction, among others [1, 2]. However, therapeutic or prophylactic efficacies of the sPLA2 inhibitors should be carefully evaluated, since gene targeting of several sPLA2s have revealed that distinct isoforms often display opposite functions in a given pathology [47]. In this sense, inhibition of multiple sPLA2s altogether could inhibit both offensive and defensive sPLA2 isoforms and thereby cancel the therapeutic effect resulting from the inhibition of the pro-inflammatory one(s), as has been seen for human rheumatoid arthritis in which a pan-sPLA2 inhibitor had no beneficial effect [74]. Thus, using an inhibitor that specifically blocks only the offensive sPLA2 may be a more desirable strategy than using the currently tested pan-sPLA2 inhibitors that block group I/II/V/X sPLA2s altogether. Nonetheless, all the above knowledge, together with application of lipidomics to in vivo systems, should help proper identification of certain PLAs and their target phospholipids or their metabolites as therapeutic targets or as novel biotherapeutic molecules in various diseases including atherosclerosis.

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