

# Secretory Phospholipase A<sub>2</sub>: A Multifaceted Family of Proatherogenic Enzymes

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Secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) represents a class of enzymes that hydrolyze phospholipids from cellular membranes and lipoproteins, resulting in multifarious proatherogenic actions in the vessel wall. Proatherogenic actions of sPLA<sub>2</sub> involve lipoprotein remodeling that facilitates proteoglycan binding and formation of lipid aggregates that are rapidly internalized by tissue macrophages. The hydrolysis of phospholipids on cell membranes generates bioactive lipids and lipoproteins with increased oxidative susceptibility. These particles and other bioactive lipids activate inflammatory pathways in various cells of the vessel wall. Transgenic mice overexpressing groups IIA, V, and X have increased atherosclerosis formation, whereas mice deficient in these sPLA<sub>2</sub> isoenzymes have less atherosclerosis formation. In apolipoprotein E knockout mice fed an atherosclerotic diet, sPLA<sub>2</sub> inhibition with varespladib reduced atherosclerosis formation. The potential for sPLA<sub>2</sub> inhibitors for preventing cardiovascular events is being investigated.

## Introduction

Various and nonredundant proatherogenic mechanisms have been identified for a subset of the secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) groups. Of the 10-member family of sPLA<sub>2</sub> enzymes, seven isoenzymes have been detected in human atherosclerotic lesions [1]. Accumulating evidence indicates that at least three members of the 10-member sPLA<sub>2</sub> family—groups IIA, V, and X—are responsible for proatherogenic actions in the vessel wall.

Members of the sPLA<sub>2</sub> family of enzymes generate bioactive lipid mediators that include lysophospho-

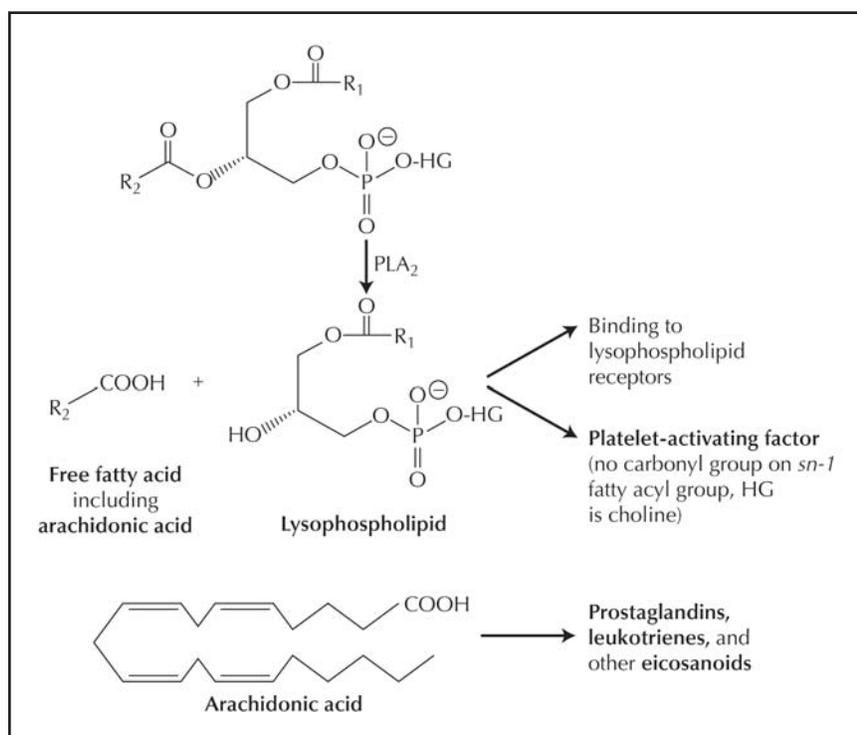
lipids, and arachidonic acid, which can be converted to eicosanoids. The bioactive lipids work together to activate multiple inflammatory processes in various cells from the arterial wall.

Group IIA sPLA<sub>2</sub> is an acute-phase protein that is expressed in various tissues and cells in response to proinflammatory cytokines, and it serves to amplify the systemic inflammatory response. In contrast to group IIA sPLA<sub>2</sub>, group V sPLA<sub>2</sub> and group X sPLA<sub>2</sub> efficiently hydrolyze phosphatidylcholine, the major phospholipid on lipoprotein surfaces. This results in smaller and more electronegative very low density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles that are avidly bound to intimal proteoglycans, where they are directly internalized by mononuclear cell-derived macrophages by pathways that are independent of conventional scavenger receptors and/or through the formation of lipid aggregates that are removed by pinocytosis.

This article reviews the contribution of the sPLA<sub>2</sub> isoenzymes involved in the augmentation of atherosclerosis. The potential role of sPLA<sub>2</sub> inhibition as a novel mechanism to reduce atherosclerosis progression and cardiovascular events is also discussed.

## Functional and Structural Characteristics of sPLA<sub>2</sub>

sPLA<sub>2</sub> represents a family of enzymes that hydrolyze the acyl ester at the *sn*-2 position of *sn*-3 glycerophospholipids to release a lysophospholipid and a free fatty acid, sometimes arachidonic acid (Fig. 1) [2]. sPLA<sub>2</sub> enzymes were given group names based on their disulfide bond pattern and on their discovery in relationship to other members of the sPLA<sub>2</sub> family [3]. Most of these proteins share a common three-dimensional structure and have molecular weights of about 16 kDa [4,5]. Group III sPLA<sub>2</sub> has a distinct structure and a molecular weight of about 55 kDa (Table 1) [2]. X-ray structures are available for human group IIA sPLA<sub>2</sub> [6] and human group X sPLA<sub>2</sub> [7]. The genes encoding group IIA sPLA<sub>2</sub> and group V sPLA<sub>2</sub> are localized in close proximity in homologous regions in human chromosome 1 and mouse chromosome 4, and these groups probably represent a gene cluster [8].



**Figure 1.** The phospholipase A<sub>2</sub> (PLA<sub>2</sub>) class of enzymes hydrolyze the *sn*-2 ester of glycerophospholipids to give a lysophospholipid and a free fatty acid. Phospholipids and lysophospholipids can have various different head groups (HG) (ie, choline for phosphatidylcholine). When the phospholipid is an *sn*-1 ether phospholipid (no carbonyl group on the top fatty acid) and when HG is choline, the lysophospholipid produced is lyso-platelet-activating factor, which can be converted to platelet-activating factor by acetylation of the *sn*-2 hydroxyl group. Lysophospholipids in general can bind to one or more cell surface receptors, which can activate various cell types. PLA<sub>2</sub> also produces a free fatty acid. When this is arachidonic acid, it can be oxygenated into a variety of different eicosanoids, including prostaglandins and leukotrienes. These, in turn, bind to cell surface receptors and activate inflammatory pathways in various cell types.

**Table 1. Structural and catalytic properties of human sPLA<sub>2</sub> groups involved in atherosclerosis**

Group	IIA	V	X
Propeptide	N (expt) <sup>*</sup>	N (expt)	Y (expt) <sup>†</sup>
Disulfides, <i>n</i>	7	6	8
N-glycosylation	N (expt)	N (expt)	Y (expt)
Extra structures	Short C-terminal		Short C-terminal
POPG μmol/ (min × mg)	220	24	14
K <sub>Ca</sub> <sup>app</sup> (μM) <sup>‡</sup>	13	1	1.5

<sup>\*</sup>N(expt) indicates no existence experimentally verified.

<sup>†</sup>Y(expt) indicates existence experimentally verified.

<sup>‡</sup>The kinetic properties of highly purified hGIII and mGIII have not yet been reported. Studies with partially purified hGIII expressed in HEK293 cells suggest that this enzyme displays a high level of sPLA<sub>2</sub> activity on phosphatidylglycerol and phosphatidylcholine vesicles typical of other highly active sPLA<sub>2</sub> enzymes (ie, group IB, IIA, IIC, IIF, V, and X sPLA enzymes).

POPG—2-palmitoyl-2-oleoyl-*sn*-glycero-3-glycerol; sPLA<sub>2</sub>—secretory phospholipase A<sub>2</sub>.

(Adapted from Lambeau and Gelb [2].)

At the cellular level, sPLA<sub>2</sub> enzymes may function during secretion (in the secretory compartment or in the extracellular space in an autocrine or paracrine manner) and after internalization. Control of sPLA<sub>2</sub> function occurs at the transcriptional level, but it is also regulated by posttranslational mechanisms during secretion, proteolytic maturation, membrane surface properties in which these enzymes operate, and degradation that is related to the interaction of the sPLA<sub>2</sub> enzyme to specific binding proteins.

Because naturally occurring phospholipids have negligible solubility in water, sPLA<sub>2</sub> enzymes must adhere to the substrate membrane interface for phospholipid hydrolysis to occur. The anchoring of sPLA<sub>2</sub> enzymes to surface phospholipids (interfacial binding) precedes binding of a phospholipid substrate in the enzyme's active site.

Electrostatic interactions of sPLA<sub>2</sub> enzymes with phospholipid vesicles represent an important aspect of interfacial binding. In the case of group IIA sPLA<sub>2</sub>, the human enzyme contains cationic residues dispersed over its surface. The large excess of positive charge over its entire exposed surface allows for this cationic enzyme to bind tightly to vesicles that contain a critical amount of anionic phospholipids (eg, phosphatidylglycerol and phosphatidylserine) [9].

Group V and X sPLA<sub>2</sub> enzymes are unique among mammalian sPLA<sub>2</sub> family members because they display relatively high affinity for anionic and zwitterionic phospholipid vesicles, whereas the other sPLA<sub>2</sub> enzymes display stronger affinity for anionic phospholipids vesicles. For example, mouse and human IIA sPLA<sub>2</sub> bind weakly to phosphatidylcholine vesicles compared with anionic vesicles, including those enriched in phosphatidylglycerol and phosphatidylserine. Thus, the various sPLA<sub>2</sub> groups have different substrate specificity that is important for lipoprotein remodeling and binding to ischemic membranes.

Secretory PLA<sub>2</sub> enzymes contain an active site slot (~ 15° Å deep) within the catalytic amino acid residues at the bottom of the slot. The region of the protein's surface that surrounds the opening of the active site slot constitutes the interfacial binding surface. The interfacial binding surface is in direct contact with the membrane when the enzyme transfers from the aqueous

phase to the membrane surface, and it serves to orient the enzyme-susceptible ester next to the catalytic residues. Tryptophan has been identified as a significant promoter of interfacial binding to anionic and zwitterionic phospholipid vesicles [10,11]. The indole side chain is thought to penetrate into the glycerol backbone region of the bilayer [10,11].

A calcium ion is bound to the catalytic site of the enzyme and directs coordination of the substrate carbonyl oxygen atoms. The concentration of calcium ion that activates the sPLA<sub>2</sub> enzyme depends on the phospholipid structure. The concentration of calcium required for the action of the entire set of mouse and human sPLA<sub>2</sub> enzymes that act on phosphatidylglycerol vesicles is in the low micromolar range [12].

### Lipoprotein Remodeling and sPLA<sub>2</sub> Enzymes

Several members of the sPLA<sub>2</sub> family of enzymes catalyze hydrolysis of surface phospholipids on lipoproteins that in turn promotes lipid accumulation in the vessel wall and cholesterol loading of macrophages or foam cell formation. sPLA<sub>2</sub>-mediated hydrolysis of VLDL, LDL [13,14], and high-density lipoprotein (HDL) phospholipids [13,15,16] promotes structural alteration in these particles, resulting in smaller lipoprotein particles. The sPLA<sub>2</sub>-modified VLDL and LDL particles transit the vessel wall more easily than unmodified particles [17,18]. Lipolysis of LDL by group IIA sPLA<sub>2</sub> [18] and group V sPLA<sub>2</sub> [19] results in conformational changes in apolipoprotein B [20] that increase LDL binding to intimal proteoglycans [21,22] and promote retention of these atherogenic lipoproteins in the vessel wall. Groups IIA and V bind to multiple heparin sulfate proteoglycans that include decorin [23], biglycan, and glypican-1 [24]. Further hydrolysis by group IIA and group V sPLA<sub>2</sub> enzymes induce reorganization of lipids, which promotes particle aggregation [19,20,25,26]. Lastly, group IIA sPLA<sub>2</sub> hydrolyzes oxidized LDL, creating a more highly oxidized LDL particle that may be cleared by scavenger receptors [27].

Group V sPLA<sub>2</sub> [28•] and group X sPLA<sub>2</sub> [29] enzymes hydrolyze VLDL, LDL, and HDL at least 20-fold more efficiently than group IIA sPLA<sub>2</sub> [30]. Hydrolysis of phosphatidylcholine from the surface of LDL generates a more negatively charged LDL particle that is efficiently incorporated into the macrophage [31]. Although group IIA sPLA<sub>2</sub> acts poorly on unmodified LDL, presumably because of its poor binding to phosphatidylcholine-rich membranes as discussed earlier, this enzyme shows enhanced ability to hydrolyze oxidized LDL. Further, sPLA<sub>2</sub>-modified HDL particles do not protect against LDL oxidation, presumably because of diminished paraoxonase activity [32]. Paraoxonase is an HDL-bound enzyme that degrades oxidized phospholipids [33]. The more highly oxidized LDL particles are cleared by scavenger receptors and contribute to foam cell formation.

### Involvement of sPLA<sub>2</sub> in Activation of Inflammatory Pathways

Of the sPLA<sub>2</sub> family, group IIA is expressed at very high levels in acute and chronic inflammatory disorders. Pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6 induce synthesis of group IIA sPLA<sub>2</sub> in arterial smooth muscle cells and hepatocytes. Multiple intracellular signaling cascades shown to regulate group IIA sPLA<sub>2</sub> expression include positive modulation in aortic smooth muscle cells by STAT3, nuclear factor- $\kappa$ B, C/EBP- $\beta$ , JAK2/STAT1 $\alpha$ , cyclic adenosine monophosphate/protein kinase A, and negative modulation by RhoA/Rho-associated kinase, RAS/MEK/ERK, p38 mitogen-activated protein kinase (MAPK), and glycogen synthase kinase-3 $\beta$  [34,35]. Group IIA sPLA<sub>2</sub>, in turn, activates signaling pathways such as MEK/ERK1/2, p38 MAPK, phosphatidylinositol 3-kinase/Akt cascades [36], and protein kinase C.

Of the sPLA<sub>2</sub> family, groups V and X act together with cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) to liberate arachidonic acid from phospholipids of mammalian cells [14,37,38]. Arachidonic acid release from the *sn*-2 position of phospholipids is the rate-limiting step in eicosanoid production. Eicosanoids include prostaglandins, thromboxanes, and leukotrienes.

Evidence that supports involvement of sPLA<sub>2</sub> in eicosanoid-mediated inflammation comes from recent studies with mouse group V and group X-deficient mice [38,39]. Human group V PLA<sub>2</sub> binds directly to the outer plasma membrane of neutrophils to release fatty acids (including arachidonic acid) and lysophospholipids (principally lysophosphatidylcholine [lyso-PC]). Meanwhile, activated 5-lipoxygenase produces leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which binds to the cell surface LTB<sub>4</sub> in an autocrine manner and triggers an MAPK cascade to rephosphorylate and reactivate cPLA<sub>2</sub>, which will then lead to an amplified and prolonged production of arachidonic acid, LTB<sub>4</sub>, and other eicosanoids. In addition to its greater potency in inducing LTB<sub>4</sub>, lyso-PC is produced in higher quantities than arachidonic acid from the outer plasma membrane of neutrophils because of the abundance of phosphatidylcholine, and therefore lyso-PC contributes more importantly to inflammatory responses. In neutrophils, cPLA<sub>2</sub> is phosphorylated by p38 MAPK, ERK1/2, or both, depending on the agonist. MAPKs are involved in the early phase of cPLA<sub>2</sub> activation, whereas ERK1/2 is involved primarily in the delayed response. Group X sPLA<sub>2</sub> has also been shown to induce arachidonic acid release by mechanisms that do not involve cPLA<sub>2</sub> [40].

The addition of exogenous group X sPLA<sub>2</sub> to cultured mammalian cells increases arachidonic acid release for cyclooxygenase-2-mediated prostaglandin E<sub>2</sub> production. The efficiency of this process is dependent on tryptophan-mediated interfacial binding to zwitterionic vesicles [41]. In an allergen-induced airway inflammation model of group X sPLA<sub>2</sub>-deficient mice, impaired CD4<sup>+</sup> and CD8<sup>+</sup> T cell trafficking occurred that was associated with

**Table 2. sPLA<sub>2</sub> proteins are present in human atherosclerotic lesions**

IIA	SMCs in intima and media Intracellularly in macrophages Extracellularly with collagen and proteoglycans in human atherosclerotic lesions
V	SMCs in media Extracellularly surrounding macrophage foam cells
X	Intracellularly in macrophage foam cells Associated with differentiated SMCs resembling myofibroblasts
SMCs—smooth muscle cells; sPLA <sub>2</sub> —secretory phospholipase A <sub>2</sub> . (Adapted from Singer et al. [12].)	

reduced T helper type 2 cell cytokine gene and protein expression and eicosanoid biosynthesis [39].

Although group IIA sPLA<sub>2</sub> bound to heparan sulfate proteoglycans has been shown to release arachidonic acid from apoptotic T cells [42], it is unclear whether this pathway contributes to downstream proinflammatory effects in other cell types. More importantly, group IIA sPLA<sub>2</sub> binds to integrins  $\alpha\beta3$  and  $\alpha4\beta1$  with high affinity at a site that is distinct from the catalytic site [43]. The binding of group IIA sPLA<sub>2</sub> to integrins induced proliferative signals mediated by ERK1/2 in cells that express integrins  $\alpha\beta3$  and  $\alpha4\beta1$ , which are expressed in various cells (including monocytes, macrophages, and endothelial cells). It has been shown that group IIA sPLA<sub>2</sub> competes with vascular cell adhesion molecule-1 (VCAM-1) binding to  $\alpha4\beta1$ . Expression of  $\alpha\beta3$  is regulated by the cytokines IL-4 and TNF- $\alpha$  and platelet-derived growth factor and fibroblast growth factor. The integrin  $\alpha\beta3$  is expressed by macrophages in early and advanced lesions. The expression of  $\alpha\beta3$  is upregulated by oxidized LDL and macrophage colony-stimulating factor [44]. The binding of group IIA sPLA<sub>2</sub> to these monocytic cells was not dependent on proteoglycan binding because these cells have very low proteoglycan content [43].

In contrast to the proinflammatory effects of group IIA sPLA<sub>2</sub>, there were no proinflammatory effects observed with group V sPLA<sub>2</sub> in a gain-of-function murine model. In group V sPLA<sub>2</sub>-overexpressing mice, there was no change in cyclooxygenase-2 expression [45]. Further, TNF- $\alpha$  and IL-6 mRNA levels were similar for mice overexpressing group V sPLA<sub>2</sub> as for control mice.

Proinflammatory effects of group X sPLA<sub>2</sub> derive from multiple pathways that include production of lysophosphatidylcholine and nonesterified fatty acids [31], and direct effects on activation of MAPK pathway, ERK1/2 kinase activation, and increased release of arachidonic acid [29]. Arachidonic acid is an important inflammatory lipid mediator that increases expression of adhesion molecules on endothelial cells, including intercellular adhesion molecule-1 and VCAM-1, which subsequently causes increased adhesion of monocytes to endothelial cells [29].

### sPLA<sub>2</sub> Isoenzymes and Atherosclerosis

Three members (group IIA, V, and X) have been detected in murine and human atherosclerotic lesions [19,31,46,47]. The distribution of sPLA<sub>2</sub> isoenzymes in the vessel wall of humans is provided in Table 2 [12]. In the next section, we review the experimental data that supports involvement of groups IIA, V, and X in atherosclerosis.

Transgenic mice constitutively overexpressing human group IIA sPLA<sub>2</sub> in various tissues developed atherosclerotic lesions on standard chow and atherogenic diets and have altered lipoprotein profiles [13]. Transplanting bone marrow cells from these transgenic mice into LDL receptor-deficient mice increased atherosclerotic lesion formation. Furthermore, human group IIA sPLA<sub>2</sub> transgenic mice with macrophage-specific overexpression showed increased lesion area and enhanced collagen deposition. Macrophage-specific overexpression of human group IIA sPLA<sub>2</sub> increased foam cell formation in LDL receptor-deficient mice (LDL<sup>-/-</sup>) transplanted with sPLA<sub>2</sub> bone marrow of transgenic mice who were fed a Western-type diet for 9 weeks [27]. The increased foam cell formation occurred despite changes in lipoprotein phospholipid distribution, plasma cholesterol concentration, or plasma sPLA<sub>2</sub> activity. In addition, group IIA sPLA<sub>2</sub> increased 12/15 lipoxygenase-mediated LDL oxidation and isoprostane 8, 12 *iso*-iPGF<sub>2 $\alpha$</sub> -VI formation. In another study, macrophage-specific expression of group IIA sPLA<sub>2</sub> increased atherosclerosis in LDL<sup>-/-</sup> mice without changing plasma cholesterol or sPLA<sub>2</sub> activity [48].

Similar to group IIA sPLA<sub>2</sub>, group V and group X enzymes are also present in human and mouse atherosclerotic lesions, but their respective distributions are variable, suggesting nonredundant function. As an example, a 4-week high-fat diet upregulates the expression of mouse group V sPLA<sub>2</sub> in aorta by fivefold, but not that of group IIA sPLA<sub>2</sub>.

Group V sPLA<sub>2</sub> has been identified as a potential factor in promoting atherosclerosis in human and murine atherosclerosis [28•]. Overexpression of mouse group V sPLA<sub>2</sub> by retrovirus-mediated gene transfer increased lesion area in LDL<sup>-/-</sup> mice, whereas mice deficient in bone marrow-derived group V sPLA<sub>2</sub> had reduced lesion area. Transgenic group V sPLA<sub>2</sub> overexpression increased lipid deposition and collagen deposition in LDL<sup>-/-</sup> mice [45]. Similar to LDL<sup>-/-</sup> mice, group V sPLA<sub>2</sub> promotes lipid deposition group in apolipoprotein (apo) E<sup>-/-</sup> mice fed a high-fat diet [49]; however, unlike the experiments in LDL<sup>-/-</sup> mice group V, deficiency did not reduce atherosclerosis in apo E<sup>-/-</sup> mice even though there was a reduction in collagen formation. These differences may relate to the increased sphingomyelin content of LDL particles isolated from apo E<sup>-/-</sup> mice, which decreases group V-mediated phosphatidylcholine hydrolysis. Group V sPLA<sub>2</sub> is expressed in human atherosclerotic lesions and human vascular cells [28•]. In human vascular cells, group V sPLA<sub>2</sub> is found extracellularly around foam cells in lipid core areas, and it is associated with smooth muscle cells in the neointima and media of intermediate and advanced lesions.

Cholesterol accumulation in the vessel wall is one mechanism for group V sPLA<sub>2</sub>-mediated atherosclerosis. In group V sPLA<sub>2</sub>-overexpressing mice, there was a decrease in LDL-sized particles after an atherogenic diet. In murine macrophages, group V sPLA<sub>2</sub>-modified LDL induced cholesteryl ester accumulation that was independent of the LDL receptor, SR-A, and CD36 scavenger receptors or the pathway that clears LDL aggregates [50]. The mechanism for the increased uptake of group V-modified LDL involves initial binding to heparan sulfate proteoglycans of the extracellular matrix, and perhaps directly binding to macrophages.

Human group X sPLA<sub>2</sub> is expressed in the intima of human atherosclerotic lesions where it colocalizes with foam cells and smooth muscle cells that resemble myofibroblasts [29]. Group X sPLA<sub>2</sub> modifies LDL particles that induce lipid accumulation in monocyte-derived macrophages. Activation of MAPK pathway and release of arachidonic acid increase expression of adhesion molecules on the endothelial surface, resulting in increased monocyte adhesion to endothelial cells.

Together, these studies suggest that human groups IIA, V, and X sPLA<sub>2</sub> have important roles in the initiation, progression, and/or rupture of lipid-rich atherosclerotic plaques by affecting plasma lipoprotein metabolism, LDL oxidation, and isoprostanes.

### sPLA<sub>2</sub> Inhibition

The effect of the sPLA<sub>2</sub> inhibition with varespladib methyl on atherosclerosis has been investigated in three animal models using apo E knockout mice [51,52••]. Varespladib sodium (sodium 2-[1-benzyl-2-ethyl-3-oxamoylindol-4-yl] oxyacetate; A-001; Anthera Pharmaceuticals, Hayward, CA, or previously LY315920; Eli Lilly & Co., Indianapolis, IN; or S5920, Shionogi & Co., Osaka, Japan) and varespladib methyl (1-H-indole-3-glyoxamide; A-002; Anthera Pharmaceuticals, Hayward, CA or previously LY333013; Eli Lilly & Co., Indianapolis, IN; or S3013, Shionogi & Co., Osaka, Japan) are small molecule inhibitors of sPLA<sub>2</sub> with specificity toward group IIA sPLA<sub>2</sub> (IC<sub>50</sub> [half maximal inhibitory concentration]: 9–14 nM), group V sPLA<sub>2</sub> (IC<sub>50</sub>: 77 nM), and group X sPLA<sub>2</sub> (IC<sub>50</sub>: 15 nM) [51]. In one study, apo E<sup>-/-</sup> mice were fed a high-fat diet for 2 weeks, and then treated with varespladib methyl or placebo for 16 weeks. Varespladib methyl treatment reduced the extent of aortic plaque coverage by 50% compared with untreated control animals [51]. In a second study of accelerated atherosclerosis induced by a combination of high-fat diet and continuous infusion of angiotensin II, the extent of aortic plaque coverage and aneurysm formation was significantly reduced after 4 weeks of treatment with varespladib methyl [51]. In a third study that investigated the potential synergy between a statin (pravastatin) and varespladib methyl to reduce atherosclerosis, apo E<sup>-/-</sup> mice were fed a Western diet for 3 months, then sacrificed and assessed for atherosclerosis [52••]. Varespladib methyl (1.5 and 150 mg/kg/d)

decreased the amount of atherosclerosis in a dose-dependent manner compared with placebo. Low dose of pravastatin (0.05 mg/d) alone resulted in a small, nonstatistically significant decrease in atherosclerosis. Combined low doses of varespladib methyl (1.5 mg/kg/d) and pravastatin caused a greater decrease in atherosclerosis than either drug when used alone, suggesting a synergistic effect. Pravastatin combined with low-dose varespladib methyl reduced plaque area by 50% [52••]. These experimental studies provide a rationale for future investigation into the role of varespladib methyl in the treatment of atherosclerosis, and the potential synergistic effect of varespladib methyl and statin therapy.

PLASMA (Phospholipase Levels and Serological Markers of Atherosclerosis) was a phase 2, randomized, double-blind, placebo-controlled, parallel-arm dose-ranging study of four doses of varespladib methyl in 396 patients with stable coronary heart disease (NCT00455546) [53••]. The primary outcome measure following 8 weeks of treatment is the change in plasma sPLA<sub>2</sub> level from baseline. PLASMA II is a randomized, placebo-controlled trial that examines the effects of once-daily dosing of varespladib methyl (250 mg, 500 mg) on sPLA<sub>2</sub> concentration, lipids, and lipoproteins in 135 patients with stable coronary heart disease (NCT00525954). In these trials, 8-week reductions in sPLA<sub>2</sub> concentration ranged by more than 90%, and reductions in LDL cholesterol ranged from 12% to 18%.

Because group IIA sPLA<sub>2</sub> is an acute-phase reactant and elevated levels of sPLA<sub>2</sub> are predictive of cardiovascular events in acute coronary syndrome patients, the potential use of an sPLA<sub>2</sub> inhibitor may provide optimal benefit for these patients. FRANCIS (Fewer Recurrent Acute Coronary Events With Near-Term Cardiovascular Inflammatory Suppression) (NCT00743925) is an ongoing phase 2 trial designed to examine the effects of varespladib methyl on plasma biomarkers in 625 patients with acute coronary syndromes who are treated with atorvastatin, 80 mg, daily. A secondary outcome is to determine differences in major cardiovascular events to generate a sample size estimate for a larger-scale cardiovascular event trial.

### Conclusions

sPLA<sub>2</sub> enzymes are involved in multiple steps in atherosclerosis that include lipoprotein remodeling, generation of proinflammatory bioactive lipids, and activation of inflammatory pathways. Transgenic mice that overexpress group IIA, group V, and group X sPLA<sub>2</sub> have all shown an increase in foam cell formation and atherosclerosis. Correspondingly, reduced atherosclerosis has been observed in loss-of-function studies for group II, group V, and group X sPLA<sub>2</sub>, and through the use of an inhibitor of sPLA<sub>2</sub> activity. Currently, the contribution of selective phospholipase A<sub>2</sub> inhibition in preventing atherosclerosis in humans remains to be established in randomized clinical trials.

## Disclosure

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Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

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