

The need for Group IIA secretory phospholipase A₂ (sPLA₂-IIA) inhibitors in inflammatory ocular disease treatment

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Abstract

The highly cationic nature of sPLA₂-IIA allows the enzyme to bind and hydrolyze an anionic phospholipid on the cell membrane, especially when damaged and stressed, causing the release of lysophospholipid and free fatty acid. These products are eventually converted into eicosanoids such as arachidonic acid, leukotrienes, and prostaglandins, leading to inflammation. Recent studies have shown that a high level of sPLA₂-IIA is associated with ocular inflammatory diseases, including dry eye disease, chronic blepharitis and allergic conjunctivitis. Therefore, inhibition of sPLA₂-IIA may serve as a promising strategy to alleviate ocular inflammation. In this review, we survey the current literature of sPLA₂-IIA on inflammatory diseases and aim to provide new insight into the study of sPLA₂-IIA inhibition for inflammatory ocular diseases.

What is sPLA₂-IIA?

All phospholipase A₂ (PLA₂) enzymes are capable of hydrolyzing the center (*sn*-2) ester bond of a natural phospholipid substrate, giving lysophospholipid and free fatty acids, including arachidonic acid [1]. However, due to variable functional and structural features including sequence, molecular weight, disulfide bonding patterns, requirement for Ca²⁺, as well as catalytic mechanisms (His/Asp, Ser/Asp or Ser/His/Asp hydrolase), evolutionary relationships, and localization [1-8], the superfamily of PLA₂ is assigned into at least fifteen separate groups and numerous subgroups [1,2,7-9]. While new groups of PLA₂ such as adipose-specific PLA₂ (AdPLA₂) have been identified, most research has focused on the five main groups: secretory PLA₂ (sPLA₂), pancreatic or cytosolic PLA₂ (cPLA₂), Ca²⁺ independent PLA₂ (iPLA₂), platelet-activating factor (PAF) acetylhydrolase (PAF-AH), and lysosomal PLA₂ [5].

The secretory PLA₂ (sPLA₂) family, originally purified from patients with rheumatoid arthritis, consists of 10 catalytic active enzymes [5,10,11]. Besides the common characteristics of the PLA₂ superfamily mentioned above, members of the sPLA₂ family share the unique features of low molecular weight, Ca²⁺ requirement for catalysis, the presence of a His/Asp dyad and six conserved disulfide bonds [2,8]. High-resolution crystal structure models revealed that two water molecules are bonded to the catalytic histidine and a nearby conserved aspartate is used as a ligand for Ca²⁺ [12]. They help to form a positively charged oxyanion hole that stabilizes the negatively charged sPLA₂ enzyme [2-4,12].

Currently, more than 11 sPLA₂ isoforms have been identified in mammalian secretions [2,5]. A few sPLA₂ isoforms are associated with disease, such as sPLA₂-IIB with obesity [13] and sPLA₂-III with atherosclerosis and colon cancer [14]. The prototypic member of the group II sPLA₂ subfamily, sPLA₂-IIa, has been shown to be induced by pro-inflammatory stimuli in a wide variety of cells and tissues of animal species as well as been associated with inflammatory, autoimmune,

allergic diseases, such as rheumatoid arthritis, asthma, septic shock, Crohn's disease, acute respiratory distress syndrome, coronary artery disease, atherosclerosis, sepsis and cancer, earning its nickname "the inflammatory sPLA₂" [10,11,15-35].

Pro-inflammatory sPLA₂-IIA

The sPLA₂-IIA enzyme is best known as a bactericide involved in the degradation of bacterial membrane thus providing a host defense mechanism against microbial infection [36-40]. The enzyme has a high affinity for anionic phospholipids and prefers binding to phosphatidylethanolamine (PE) than phosphatidylcholine (PC), which are found abundantly on bacterial membranes [37]. Due to its highly cationic nature, sPLA₂-IIA binds tightly to the anionic phospholipids on Gram-positive bacteria, allowing the enzyme to penetrate the cell wall and hydrolyze membrane phospholipids, causing bacterial death [41].

Under physiological conditions in mammalian membranes, the inner leaflet contains anionic phosphatidylserine (PS) and PE while the exposed outer leaflet contains neutral phospholipids, such as PC, allowing for relatively low levels of sPLA₂-IIA enzymatic activity [42-45]. When the phospholipid distribution is perturbed by stress from the oxidation of phospholipids, increased levels of anionic PS and PE are transported to the outer leaflet, which activates sPLA₂-IIA leading to indiscriminate hydrolysis of outer leaflet phospholipids and inflammation [46-50].

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The activation of its enzymatic function is triggered by environmental stress or diseased conditions, promoting release of arachidonic acid (AA) and the cyclooxygenase (COX)-mediated production of prostaglandin (PG) (Figure 1) [51-57]. Once AA is released, biosynthesis of a multitude of eicosanoids, including prostaglandins, such as prostaglandin E₂ (PGE₂), thromboxanes and leukotrienes is initiated through either the external plasma membrane pathway or heparan sulfate proteoglycan (HSPG)-shuttling pathway with the help of cyclooxygenase and lipoxygenase enzymes [52,58-60]. The newly synthesized eicosanoids then bind to specific G-protein coupled receptors, activating multiple signaling transduction pathways that lead to acute or chronic inflammation or even cancers [61-65]. In addition, the lysophospholipids generated by sPLA₂-IIA hydrolysis also serve as precursors for lipid mediators, such as platelet activating factor (PAF), which lead to inflammation [66]. Since spontaneous AA release hardly occurs in unstressed human cells, activating sPLA₂-

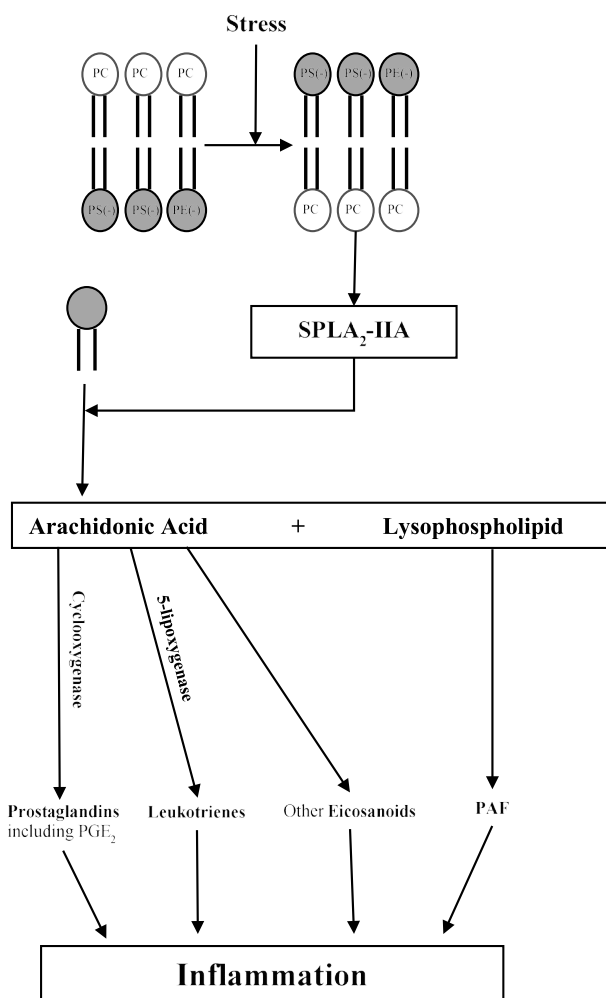


Figure 1. sPLA₂-IIA activation promotes inflammation. The oxidation of phospholipids produces stress causing anionic phospholipids (phosphatidylserine (PS) and phosphatidylethanolamine (PE)) to be transported to the outer leaflet, activating cationic sPLA₂-IIA. Higher sPLA₂-IIA activity promotes the hydrolysis of outer leaflet phospholipids into arachidonic acid and lysophospholipids. Through cyclooxygenase and 5-lipoxygenase enzymes, arachidonic acid is converted into prostaglandins, leukotrienes, and other eicosanoids. Lysophospholipids is converted into platelet-activating factors (PAFs). All four products have been linked to inflammation. Many inflammatory treatments target the arachidonic acid pathway and its enzymes, but targeting sPLA₂-IIA may offer direct inhibition to the formation of all downstream inflammatory products

IIA is the critical first step of the inflammatory cascade and therefore inhibiting sPLA₂-IIA will ultimately prevent the downward formation of additional inflammatory products [67].

sPLA₂-IIA in tears and inflammatory ocular diseases

High levels of sPLA₂-IIA activity have been found in the progression of non-ocular inflammatory diseases and cancers, such as atherosclerosis [26,27], astrocytoma [28], colorectal cancer [29], lung cancer [30-32], prostate cancer [33], rheumatoid arthritis [34] and acute respiratory distress syndrome (ARDS; [35]) as well as many inflammatory ocular diseases. Since phospholipids serve as surfactants between the aqueous and oil layers of the tear film, higher levels of sPLA₂-IIA disrupt the phospholipid layer causing the production of eicosanoids in tears and potentially chronic blepharitis, a condition characterized by the inflammation of the eyelid margins [1,68,69]. In addition, an increase in the number of free fatty acid and sPLA₂-IIA have been found on contact lenses of patients with contact lens intolerance, emphasizing sPLA₂-IIA's ability to damage the ocular surface, which leads to discomfort and dryness [70,71].

In allergic conjunctivitis (AC), an eye inflammation caused by an allergic reaction to substances, such as pollen, sPLA₂-IIA acts as a chemoattractant for eosinophils, which have been activated by allergens irritating the conjunctiva [72]. Higher levels of sPLA₂-IIA create tear film instability, leading to the further progression of allergic conjunctivitis [73, 74]. In patients with dry eye disease (DED) or keratoconjunctivitis sicca, a disease noted by aqueous tear deficiency, high levels of sPLA₂-IIA activity and protein concentration stimulate PGE₂ production in compromised ocular surface epithelial cells, resulting in the excess production of cytokines in tears and inflammatory cell infiltration [75-78].

Importance of sPLA₂-IIA inhibition

The most commonly prescribed drugs for treatment of ocular inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase, thereby suppressing arachidonic acid metabolism and the synthesis of prostaglandins as seen in Figure 1 [79,80]. However, previous studies have shown the possibility of corneal melting and perforation following the use of NSAIDs after ocular surgery in patients with or without systemic diseases, such as rheumatoid arthritis, Sjögren Syndrome, and rosacea [81-83]. Since NSAIDs only inhibit COX-1 and COX-2, having no effect on leukotrienes or PAF [84], inhibiting sPLA₂-IIA offers a better solution in blocking the production of additional inflammatory substances.

Furthermore, a natural inhibitor of sPLA₂-IIA, ochnaflavone has been shown to strongly inhibit sPLA₂-IIA activity by preventing the progression of calcium-tetrachloride (CCl₄)-induced PE hydrolysis [85]. Since several ocular inflammatory diseases, such as chronic blepharitis [68] involve the degradation of the tear film, implicated by sPLA₂-IIA-initiated PS hydrolysis, inhibiting sPLA₂-IIA may be a promising alternative to prevent the formation of inflammatory mediators and the progression of inflammatory ocular diseases.

The search for effective inhibitors with high specificity to sPLA₂-IIA

One of the first small-molecule inhibitors specific for human sPLA₂-IIA, LY311727, was designed to fit the active site of human non-pancreatic sPLA₂ enzyme through tight binding interactions, in contrast to the weak or lack of inhibition of previously used snake venom and

pancreatic enzyme derivatives [86-88]. While LY311727 has a high affinity for group II sPLA₂ at an enzyme concentration that is 20,000 times less than the phospholipid substrate concentration, studies have found that the inhibitor can also inhibit group V sPLA₂ with an IC₅₀ of 36 nanomolar (nM) [79,89], stressing one of the main obstacles in sPLA₂-IIA inhibition research, sufficient sPLA₂-IIA selectivity.

The most heavily researched sPLA₂ inhibitor, Varespladib sodium (also known as LY315920 and S-5920) was optimized to be five to ten-folds less active against sPLA₂ group V and 40-fold less active against group IB, with negligible activity against groups IV, VI and cytosolic phospholipases [90-92]. The small-molecule indole-based sPLA₂ inhibitor has IC₅₀ as low as 9-14 nM for human sPLA₂-IIA [93] and was found to mechanistically inhibit surfactant degradation [94]. Having wide existing pharmacological data, Varespladib has already undertaken phase II and III clinical studies in adults (both orally and intravenously) for a variety of clinical symptoms, such as sepsis, acute coronary syndrome, and sickle cell disease-induced acute chest syndrome (IMPACTS trial, NCT00434473) [9,95-100]. However, the oral pro-drug of Varespladib sodium, Varespladib-methyl (LY333013) has been found to have varying efficacies in the treatment of coronary artery disease (CAD) [96-98] as well as been ineffective in treatment of rheumatoid arthritis, leading researchers to believe that Varespladib has limited efficacy in clinical studies [101].

One reason for this failure may be due to the incomplete inactivation of sPLA₂ influenced by an inadequate inhibitor concentration in the trial, which the authors fail to report [102]. In some diseases, Varespladib was found to bind non-specifically to high levels of proteins before reaching the sPLA₂ catalytic site for effective inhibition [91,100]. Increasing Varespladib dosage has also been noted as a solution to overcoming incomplete inactivation of sPLA₂ [100]. Furthermore, an alternative small molecule sPLA₂ inhibitor (PIP-18) was proposed to have a stronger suppressive effect on sPLA₂ transcription and translation than Varespladib which offers another solution to the limited efficacy found in Varespladib clinical studies [103,104]. This 18-residue peptide contains two presumed pharmacophores. Each pharmacophore binds to more than one molecule of sPLA₂ through the hydrophobic binding pocket near the N-terminal helix of sPLA₂, inhibiting more than 70% of sPLA₂ secreted and lowering PGE₂ production [103-106].

On the other hand, a naturally produced triterpene, Celastrol, has shown multiple inhibitory effects on sPLA₂-IIA and other enzymes along the AA pathway including COX-2 and 5-LOX, in an apparent concentration-dependent manner [107]. The inhibitor demonstrates positive pharmacological effects and use as a therapeutic molecule in inflammatory diseases, such as arthritis and cancer [108], suggesting that targeting both sPLA₂-IIA and inflammatory mediators may serve as a useful and potentially powerful strategy.

Human integrins αvβ3 and α4β1 on leukocytes have also recently been implicated as two sPLA₂-IIA human receptors and targets for chronic inflammatory diseases [109-111]. Using docking simulation, Takada et al. designed a peptide, named compound 21, which was able to inhibit nearly 93% of sPLA₂-IIA-integrin αvβ3 interactions in water soluble conditions thus suppressing αvβ3-mediated cell adhesion, migration and inflammation [112]. In addition, other inhibitors with heterocycle-peptide conjugate structures have been identified, such as compound 4, compound 8 and compound 16, which binds to integrins and blocks the ligand function of sPLA₂-IIA [110]. This type of inhibitor may provide a different and effective strategy to suppressing sPLA₂-IIA-induced inflammation.

sPLA₂-IIA inhibitors for symptom relief in ocular inflammatory diseases

There is an increasing body of evidence demonstrating the beneficial use of sPLA₂-IIA inhibitors in ocular inflammatory diseases. Oleonic acid was found to effectively reduce production of sPLA₂-IIA and other pro-inflammatory mediators by eosinophils and mast cells in the conjunctiva of allergic mice [74]. Varespladib and a recently studied sPLA₂-IIA inhibitor, S-3319, have also shown to decrease symptoms of corneal superficial punctate keratitis (SPK), a disorder noted by the death of small group of cells in DED mice [113]. S-3319 was shown to selectively bind to sPLA₂-IIA in vivo in mice with an IC₅₀ as high as 29 nM [114-116] as well as in mouse conjunctiva [77,117]. S-3319 also has a significant inhibitory role on PGE₂ production in the conjunctiva of BALB/c mouse models, used for their functional sPLA₂-IIA gene [77,118]. Like Varespladib, increasing dosage (0.08μM to 10μM) reduced PGE₂ production by 29% [77], indicating that current sPLA₂-IIA inhibition may have low potency. All three inhibitors decrease symptoms in conjunctival or corneal epithelia, suggesting their involvement with sPLA₂-IIA activity and/or production. However, the current basic research on sPLA₂-IIA in ocular inflammatory diseases is limited to only a few inhibitors (Table 1) and future research will need to experiment with inhibitors used in other inflammatory diseases to possibly combat issues of potency and high IC₅₀.

Concluding remarks

sPLA₂-IIA is confirmed to be higher in concentration and activity in ocular inflammatory diseases, such as DED, allergic conjunctivitis, and chronic blepharitis, which demonstrates a need for sPLA₂-IIA inhibition in treatment of inflammation on the human ocular surface. However, due to the multiple mechanisms of action of sPLA₂-IIA enzymes as well as the limited efficacy of inhibitors in clinical trials, the usefulness of inhibiting sPLA₂-IIA has not yet been realized. Basic research in sPLA₂-IIA inhibition with tissue culture and the ocular surface of animal models will continue to be effective tools leading to potential effective inhibitors and clinical studies in human diseases. Future research will need to focus on identifying novel or existing sPLA₂-IIA inhibitors with higher affinity for sPLA₂-IIA, as well as determining the mechanism of sPLA₂-IIA in ocular inflammatory diseases. Only then will designing new inhibitors, with methods such as docking simulation, as well as specific humanized and sPLA₂-IIA neutralizing antibodies for the study of inhibition be effective. Consideration must also focus on sPLA₂-IIA

Table 1. List of inhibitors of sPLA₂-IIA used in inflammation studies

Number	Inhibitor	IC ₅₀	Used in ocular inflammatory studies	Reference
1	Oleonic Acid	-	Yes	[74]
2	Ochnaflavone	3.45 μM	No	[85]
3	LY311727	36 nM	No	[79,89]
4	LY315920/LY333013	9 – 14 nM	Yes	[93,113]
5	PIP-18	1.19 μM	No	[103-106]
6	Celastrol	-	No	[107]
7	Compound 4	85 μM	No	[110]
8	Compound 8	20 μM	No	[110]
9	Compound 16	-	No	[110]
10	Compound 21	71 μM	No	[112]
11	S-3319	29 nM	Yes	[114-116]
12	Fucoidan	-	No	[119]
13	KH064	29 nM	No	[120]
14	PX-18	-	No	[121]
15	Ursolic Acid	12 – 18 μM	No	[122]

inhibitors with high water solubility to allow for dissolution on the ocular surface with high potency. Identifying effective and selective sPLA₂-IIA inhibitors will provide the key to future approaches in the treatment of ocular surface inflammation.

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