REVIEW ARTICLE

Phospholipase A2 is an Inflammatory Predictor in Cardiovascular Diseases: Is there any Spacious Room to Prove the Causation?

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Abstract: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme family of phospholipase A2 produced by the inflammatory cell in atherosclerotic plaque. It is transported in the circulation, attached mainly to low-density lipoprotein-cholesterol (LDL-C). It hydrolyzes glycerophospholipids particularly fatty acids at the sn-2 position and produces numerous bioactive lipids; and leads to endothelial dysfunction, atherosclerotic plaque inflammation, and development of the necrotic core in plaques.

There are two kinds of phospholipase A2, namely: secretory phospholipase A2 (sPLA2) and Lp-PLA2. They are deemed as evolving predictors of cardiovascular disease (CVD) risk in hospital- and population-based studies, including healthy subjects, acute coronary syndromes (ACS) and patients with CVD. Unfortunately, Lp-PLA2 inhibitor (darapladib) and s-PLA2 inhibitor (varespladib methyl) failed to prove to lower the risk of composite CVD mortality, myocardial infarction and stroke in those with stable CVD and ACS.

Herein, we describe the explanation based on the existing data why there is still a discrepancy among them. So, it highlights the opinion that phospholipase A2 is merely the inflammatory biomarkers of CVD and playing an important role in atherosclerosis. Further, there is more spacious room to prove the causation.

Keywords: Lipoprotein-associated phospholipase A2, LDL-C, cardiovascular disease, acute coronary syndromes, myocardial infarction, atherosclerosis.

1. INTRODUCTION

Cardiovascular disease (CVD) is a rising public health problem throughout Asia and the Middle East. Moreover, CVD is mainly among the most dominant and devastating illnesses [1]. Dyslipidemia is a vital risk factor in the initiation of atherosclerosis and related cardiovascular outcomes; consequently, efficacious therapeutic strategies which are significant in lowering the risk of CVD are needed [2]. Developing countries contribute around 80% of the global CVD death [2, 3], however, the CVD mortality has been declining in the last decade in developed countries. It is recognized that dyslipidemia is a significant CVD risk factor, in which the failure to achieve significantly targeted lipid levels leads to the residual risk of CVD [4].

The pathophysiology of CVD is underpinned by atherosclerosis. The process is initiated by trapping oxidized low-density lipoprotein-cholesterol (LDL-C) in the sub-intimal space of large and medium-sized arteries. Oxidized LDL-C (OxLDL) is a general term which includes various oxidative modifications to LDL lipid moieties and apolipoprotein B (ApoB), the structural protein of the LDL-C particle [5]. These modifications consist of restructuring of the phospholipid molecules with exposure of phosphorylcholine and aduction of aldehydes, namely malondialdehyde (MDA), to ApoB. The enzyme called phospholipase A2 modifies the oxidative changes in LDL-C.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is produced by inflammatory cells in atherosclerotic plaque and is categorized as an enzyme family of phospholipase-A2. It is transported in the circulation and is mainly attached to LDL-C. The Lp-PLA2 and another phospholipase A2 (such as secretory phospholipase A2-sPLA2) proliferate the inflammatory reaction by generating precursors of a mass that are significantly related with CVD events in stable coronary heart diseases (CHD), acute coronary syndromes (ACS), and incidental peripheral arterial diseases (PAD) [6-8]. In a meta-analysis, there is a continuous association between the activity of plasma Lp-PLA2 and CHD risk. There is also a relative increase in the risk of 1.10 (95% CI, 1.05 to 1.16) for each 1-
SD increase in plasma Lp-PLA₂ activity after being adjusted for the traditional CVD risk factors [9]. Thus, the existing evidence has inspired endeavors to develop phospholipase inhibitors: agents to prevent CVD.

Darapladib is a molecule and a potent oral Lp-PLA₂ inhibitor, and varespladib methyl is another nonspecific pan-secretory phospholipase A₂ inhibitor. In an atherosclerotic animal model, darapladib reduces the expression of Lp-PLA₂ in atherosclerotic plaque, decreases the necrotic size within the plaque, and inhibits the lesion initiation in coronary arteries [10]. It reduces the Lp-PLA₂ activity in human carotid plaque as well [11]. Another study demonstrates that varespladib methyl reduces the concentrations of sPLA₂-IIA by more than 90%, also reducing the LDL-C and C-Reactive Protein (CRP) in those with stable coronary artery disease and ACS [12, 13].

Although these drugs have demonstrated their role in improving the atherosclerotic process, unfortunately, in subjects with stable CHD, darapladib does not considerably decrease the primary composite outcomes of cardiovascular mortality, myocardial infarction, or stroke [14]. Likewise, varespladib methyl does not have the clinical benefit of decreasing recurrent cardiovascular outcomes and, even further, considerably increases myocardial infarction [15]. This review article elaborates the role of Lp-PLA₂ and sPLA₂-IIA in atherosclerosis and CVD, as published in the literature over the last 25 years, until 2019, their biological activity, and whether there is an inconsistency in their clinical benefits.

2. ROLE OF PHOSPHOLIPASE A₂ IN ATHEROSCLEROSIS

The phospholipase A₂ superfamily of enzymes has been recognized to have a role in atherosclerosis, and at least two groups in this family of enzymes are deemed potential candidates for CVD prevention. Human biomarker studies, animal studies, imaging studies, and genome-wide atherosclerosis studies have presented the rationale for clinical outcome trials directed at inhibiting sPLA₂-IIA and Lp-PLA₂ [16]. One study suggests that plasma sPLA₂ is associated with ACS via increased serum amyloid-A protein, representing an inflammatory response in ACS [6].

The phospholipase A₂ superfamily enzymes are considered to have the capacity to hydrolyze fatty acids at the sn-2 position of glycerophospholipids and create plentiful of bioactive lipids (Fig. 1) [16]. Plasma levels and activity of two families of phospholipase A₂ enzymes, namely, sPLA₂-IIA and Lp-PLA₂, have been assessed as biomarkers of CVD risk in hospital-based and population-based studies including apparently healthy subjects, subjects with ACS, and patients with established CHD [6, 9, 17, 18]. Moreover, a multivariate analysis reveals that high sPLA₂-IIA mass and activity levels as an independent predictor of early atherosclerosis in metabolic syndrome (Mets) subjects [19]. Even the Lp-PLA₂ concentrations in human plasma are associated with the CHD severity, and an apparent interaction between Lp-PLA₂ and classical CVD risk factors are settled in predicting CHD [20].

Lipoprotein-associated phospholipase A₂, a vascular-specific inflammatory biomarker, is an enzyme produced by macrophages, T-lymphocytes, and monocytes, as well as the mast cells in atherosclerotic plaques, principally within the necrotic core and fibrotic cap of vulnerable plaques [21-24]. Lp-PLA₂ hydrolyzes the modified and oxidized phospholipids on the LDL-C surface [24] and within the plaques to lead to endothelial dysfunction [25], plaque inflammation, and formation of the necrotic core in atherosclerotic plaques. It releases arachidonic acids, lysophosphatidylcholine, and oxidized fatty acids. This process is well-recognized as a trigger of the inflammatory reaction.

Fig. (1). Phospholipase A₂ enzymatic activity generates lipid products with biological properties and functions [16]. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
Further, Lp-PLA₂ is hypothesized to take an essential role in oxidative modification of LDL-C and initiation of inflammatory reactions in the arterial intima [23, 26, 27]. The congregation of lysophosphatidylethanolamine and oxidized fatty acids in the sub-intimal space contributes to the development of the plaque lipid "core." Furthermore, the macrophages, once they ingest these substrates, would consequently advance their conversion into foam cells. Additionally, lysophosphatidylethanolamine provokes the creation of reactive oxygen species such as superoxide by triggering the endothelial nicotinamide adenine dinucleotide phosphate oxidase and by activating nitric oxide synthase (eNOS) “uncoupling” [28]. The further process would drive the enzyme to become a superoxide and peroxynitrite producer. Consequently, this contributes to atherosclerosis and plaque destabilization. Serum levels of Lp-PLA₂ change considerably during the early phase of ACS, and augmented Lp-PLA₂ independently predicts CVD outcomes in patients with ACS after adjustment for potential confounders [29]. Elevated Lp-PLA₂ concentrations in the elderly Chinese subjects were associated with recurrent vascular events, a higher risk of carotid atherosclerosis and myocardial infarction and CVD death [30].

Therefore, experimental pieces of evidence indicate that Lp-PLA₂ is involved in multiple stages of atherosclerosis due to its pro-inflammatory and pro-oxidative effects (Fig. 2) and may be a useful biomarker to predict the clinical outcomes of patients with CHD independent of traditional CVD risk factors. The Lp-PLA₂ activity and mass, each demonstrates the continuous associations with the risk of CHD, similar with non-high density lipoprotein cholesterol or systolic blood pressure [19]. In patients with TIA (transient ischemic attack) and first ischemic stroke, elevated Lp-PLA₂ activity levels are associated with recurrent vascular events. Significant associations are also encountered between high hs-CRP and plasma Lp-PLA₂ concentration and carotid stenosis (OR: 2.62, 95% CI: 0.93 - 7.38). This result suggests that the combination of hs-CRP and plasma Lp-PLA₂ would be better predictors than either protein alone concerning carotid atherosclerosis [31].

Also, in the general population, high plasma Lp-PLA₂ levels are related to the risk of stroke [32]. While a higher Lp-PLA₂ activity is related to an enhanced risk for incident PAD, it is more likely that traditional CVD risk factors are more causative [7]. However, another study confirms no evidence of an association between Lp-PLA₂ and incident PAD [33]. Additionally, Lp-PLA₂ takes part by a critical role in microvascular dysfunction and oxidative stress, showing a positive relationship with metabolic disorders [34]. This result is translated to another study, indicating that higher plasma Lp-PLA₂ concentrations are related to increasing the risk of mortality and the incident of diabetic retinopathy in diabetic subjects [35]. Further, Lp-PLA₂ and sPLA₂ are closely associated with insulin resistance and macroangiopathy in diabetic patients [36]. Accordingly; the phospholipase A₂ is considered to modify the micro- and macroangiopathy complications in those with diabetes mellitus. Plasma Lp-PLA₂ activity is also discovered to be higher in subjects with definite familial hypercholesterolemia (FH) than in non-definite FH subjects, independently of LDL-C concentrations and statin consumption. This confirms that FH patients present higher arterial inflammation, which may contribute to a higher CVD risk [37].

3. STRUCTURE OF Lp-PLA₂:

Plasma Lp-PLA₂ consists of a 43-kDa-monomer polypeptide, and the PLAC2G7 gene encodes this enzyme [38].

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Fig. (2). Pathogenic role of lipoprotein-associated phospholipase A2 in atherosclerosis development [25]. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
Lp-PLA₂ binds to two separate domains on the ApoB. Approximately 70% of this enzyme is associated with LDL-C and lipoprotein (a) once it is secreted in the plasma [39, 40], and the remainder is associated with high-density lipoprotein cholesterol (HDL-C). Previously, plasma Lp-PLA₂ is also named as a platelet-activating factor acetylhydrolase (PAF-AH) (Fig. 3). This enzyme is a heterotetrameric enzyme consisting of catalytic 29- and 30-kDa and non-catalytic 45-kDa subunits (Fig. 4).

It is initially a purified enzyme that hydrolyzes the acetyl group attached to the sn-2 position of the platelet-activating factor (PAF) [41]. Consequently, it is regarded as having anti-inflammatory and anti-atherogenic properties as well.

Beyond this, there is a dissociable and a non-dissociable form of the plasma Lp-PLA₂ [42]. The activity of plasma Lp-PLA₂ in vivo is affected by the transition between them, and it is considered to be the novel proposed mechanism. The amino acid sequence of human plasma PAF-AH involves 441 amino acid residues in length and contains a Gly-X-Ser-Gly (GXXG) motif, which is also found in esterase, lipase, and other members of the α/β hydrolase superfamily of enzymes [38, 43]. The crystal structure of Lp-PLA₂ has a classic α/β serine hydrolase fold containing a catalytic triad Ser273, Asp296, and His351 [43, 44]. Another triad (Trp115, Leu116, and Tyr205) is involved in the interaction with LDL-C [45].

4. GENETICS OF Lp-PLA₂

The plasma Lp-PLA₂ is encoded by the PLA2G7 gene. This gene is positioned in chromosome 6p21.2 to 12 and entails 12 exons. Its cDNA has been cloned in 1995 [43] and contains an open reading frame codifying a precursor of 441 amino acids that is cleaved into 45.4 kDa mature protein [46]. Heritability studies revealed that approximately 62% of the Lp-PLA₂ activity variation was deemed to be a genetic factor [47]. Traditional CVD risk factors and genetic difference contributed to variability in Lp-PLA₂ activity and concentration [48].

The PLA2G7 gene is typified by the non-synonymous polymorphisms which could produce a loss of function in the enzymatic activity [24]. As stated by one study and a recent meta-analysis, Caucasian has a higher Lp-PLA₂ activity level than Hispanics and African-Americans. It justifies the conclusion that Lp-PLA₂ is genetically influenced [49, 50]. By contrast, atherosclerosis [51], stroke, [52] and dilated cardiomyopathy [53] are associated with a point mutation (rs76863441) close to the active site of the enzyme [54], which is found in 4% of Japanese people and implies undetectable plasma LpPLA₂ activity. Likewise, natural insufficiency in LpPLA₂ activity due to polymorphism in PLA2G7 gene (279F allele) protects from CHD in a Korean population [55].

Nevertheless, the association between A379V polymorphism and enzymatic levels and CVD outcomes was diverse amongst the studies, with some of them presenting the V379 allele as associated with increased enzyme levels [56, 57] whereas the other studies exhibited lower levels [58]. Also, a recent metaanalysis result proposed that V279F polymorphism in PLA2G7 gene had a protective effect on CVD, while R92H polymorphism might contribute toward the enhanced risk of clinical atherosclerosis [59] (Figs. 5 and 6).

Furthermore, Lp-PLA₂ activity was proven to be 10% lower in female subjects in comparison with male, perhaps due to the higher estrogen concentration in women, down-
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**Key Points**

- **Regulating Lp-PLA₂ activity and reducing LDL-C concentration.** All this evidence contributes further to discrepancies in the theorized causative relationship between Lp-PLA₂ and CVD.

**5. EVIDENCE FROM RANDOMIZED CLINICAL TRIALS**

Darapladib is a selective, effective and reversible oral agent inhibiting plasma Lp-PLA₂ [60] that has also been shown to reduce Lp-PLA₂ activity in human carotid plaque [11]. Furthermore, darapladib ceases the progression of the necrotic core of coronary plaques, compared to placebo. This evidence is shown in the Integrated Biomarker and Imaging Study 2 (IBIS-2) [61]. The mechanism of darapladib in halting the atherosclerotic progression has been verified in an animal study using the Sprague-Dawley rat model; this study signified that darapladib reduced the foam cells number, inducible nitric oxide synthase (i-NOS), and intracellular adhesion molecule-1 (ICAM-1) expression in the aorta at the early stages of atherosclerosis in type-2 diabetic model [62]. Consequently, there is a suggestion that altering the composition of atherosclerotic plaques to a less vulnerable state might decrease the risk of CVD outcomes.

The above hypothesis-generating statements have been further challenged to be translated using clinical endpoints. In the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial [14], darapladib, unfortunately, did not significantly show the clinical benefits of primary composite endpoints of cardiovascular mortality, myocardial infarction or stroke decrement, in those with stable CHD. This was a large, multicenter, randomized clinical study, randomly assigning 15,828 subjects with stable CHD. The median follow-up of the study was of 3.7 years. The absence of clinical effects of this drug on the primary CVD outcomes might be related to a minor impact on unstable atherosclerotic plaque than was expected by the earlier studies. The CVD risk of all patients in that trial had been minimized by standard concurrent therapy. Over a third of the patients had an LDL cholesterol concentration lower than 70 mg/dL at baseline; moreover, approximately 75% of the patients underwent revascularization procedures. Another point was that 96% of the patients in the study was consuming statins. Statins consumption has been revealed to decrease the concentration of plasma Lp-PLA₂ by up to 35% [63]. The long-term inhibition of endogenous Lp-PLA₂ activity with darapladib was not associated with a change in plaque progression, vulnerability indices after six months of therapy, or coronary endothelial function improvement [64]. These studies suggested that the endogenous Lp-PLA₂ pathway might not have a direct, independent role in the progression of early atherosclerosis in humans [65].

Other explanations might come from the current meta-analysis [59], which disclosed a significant negative association between V279F polymorphism and clinical atherosclerosis. A rare, non-synonymous polymorphism (V279F) was...

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**Fig. (5).** V279F polymorphism of PLA2G7 gene in Asian and Caucasian population [60]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

**Fig. (6).** R92H polymorphism of PLA2G7 gene in Asian and Caucasian population [60]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*
commonly encountered in Japanese, Turk, Kyrgyz, and Azerbaijani populations. These were associated with a decreased enzymatic level of Lp-PLA₂ in heterozygous subjects and complete loss of enzymatic levels in homozygous subjects [66]. All the above evidence may affect the results of those clinical outcome trials, in contrast to those of the previous mechanistic studies.

Another phospholipase inhibitor drug, varespladib methyl, is a nonspecific pan-sPLA₂ inhibitor with positive effects on atherosclerotic plaques in animal studies. Previous studies confirmed that varespladib methyl decreased the concentration of sPLA₂-IIA over 90% in addition to lowering LDL-cholesterol and C-reactive protein in stable CHD and ACS patients. The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) trial was planned to evaluate the clinical benefits of varespladib methyl on cardiovascular risk in patients with ACS [15].

Although experimental and observational clinical studies suggest that pan-inhibition of plasma sPLA₂ will provide beneficial CVD outcomes and significantly reduce the post-ACS inflammatory response [13, 67], the VISTA-16 trial shows evidence contrary to this. The VISTA-16 trial demonstrated that in those with current ACS, varespladib methyl did not decrease the risk of recurrent CVD events and even significantly enhanced the myocardial infarction risk. Hence, the authors concluded that sPLA₂ inhibition with varespladib methyl could be harmful and is not a valuable strategy to reduce adverse CVD outcomes after ACS [15]. Possible factors in this negative result include insufficient penetration of varespladib methyl into vascular cells to hinder pro-inflammatory intracellular mediators. Otherwise, varespladib methyl might have abolished the effects of both pro-atherogenic (IIA and V) and anti-atherogenic sPLA₂ isoforms. The above results with sPLA₂ inhibition highlight how the identification of a plasma biomarker of CVD risk does not automatically suggest that pharmacologic inhibition of the biomarker will diminish the cardiovascular risk. The failure to prove any clinical advantage has been supported by a current report from Mendelian randomization studies and reveals that sPLA₂ does not independently take a causal role in CVD [68]. Despite strong biological plausibility and convincing evidence from multiple studies and the failure of Mendelian randomization studies to prove the evidence of causation, this ideally should support the identification of the novel biomarkers most likely to be causally implicated in the pathobiology of CVD, shed light on convincing therapeutic targets and, finally, mitigate the huge costs expended in clinical trials [69].

None of the phospholipase inhibitors in those clinical trials have been distributed to the market yet. The failure of the Lp-PLA₂ inhibitor darapladib currently suggests that this enzyme is just a biomarker of vascular inflammation rather than playing a key role in the causal pathway of CVD. These findings, together with the failure of the sPLA₂ inhibitor varespladib methyl for the treatment of CVD, may indicate that more in-depth knowledge of these enzymes is needed.

CONCLUSION

Deciphering all the data together, Lp-PLA₂ and sPLA₂ are merely the biomarkers of vascular inflammation and play an important role in atherosclerosis. These may indicate that a more in-depth study is needed to prove the causation.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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