New Insights Into the Role of Lipoprotein(a)-Associated Lipoprotein-Associated Phospholipase A2 in Atherosclerosis and Cardiovascular Disease

Sotirios Tsimikas, Loukas D. Tsironis, Alexandros D. Tselepis

Abstract—Lipoprotein(a) [Lp(a)] plays an important role in atherosclerosis. The biological effects of Lp(a) have been attributed either to apolipoprotein(a) or to its low-density lipoprotein-like particle. Lp(a) contains platelet-activating factor acetylhydrolase, an enzyme that exhibits a Ca\(^{2+}\)/H\(_{11001}\)-independent phospholipase A\(_{2}\) activity and is complexed to lipoproteins in plasma; thus, it is also referred to as lipoprotein-associated phospholipase A\(_{2}\). Substrates for lipoprotein-associated phospholipase A\(_{2}\) include phospholipids containing oxidatively fragmented residues at the sn-2 position (oxidized phospholipids; OxPLs). OxPLs may play important roles in vascular inflammation and atherosclerosis. Plasma levels of OxPLs present on apolipoprotein B-100 particles (OxPL/apolipoprotein B) are correlated with coronary artery, carotid, and peripheral arterial disease. Furthermore, OxPL/apolipoprotein B levels in plasma are strongly correlated with Lp(a) levels, are preferentially sequestered on Lp(a), and thus are potentially subjected to degradation by the Lp(a)-associated lipoprotein-associated phospholipase A\(_{2}\). The present review article focuses specifically on the characteristics of the lipoprotein-associated phospholipase A\(_{2}\) associated with Lp(a) and discusses the possible role of this enzyme in view of emerging data showing that OxPLs in plasma are preferentially sequestered on Lp(a) and may significantly contribute to the increased atherogenicity of this lipoprotein. (Arterioscler Thromb Vasc Biol. 2007;27:2094-2099.)

Key Words: atherosclerosis ■ lipoprotein(a) ■ lipoprotein-associated phospholipase A\(_{2}\) ■ oxidized phospholipids ■ platelet-activating factor acetylhydrolase

Lipoprotein(a) [Lp(a)] is composed of a low-density lipoprotein (LDL)-like particle to which a large, highly glycosylated apolipoprotein(a) [apo(a)] is linked by a single disulfide bridge. Plasma Lp(a) levels vary widely between individuals and are largely determined by their apo(a) size, to which they are inversely related. Apo(a) is highly polymorphic because of a variable number of tandemly repeated copies of a motif resembling kringle IV (type 2 repeats) of plasminogen.\(^{1}\)

The physiological role of Lp(a) is largely unknown. However, Lp(a) is recognized as an independent risk factor for atherosclerotic cardiovascular disease, although the mechanisms by which it contributes to atherosclerosis remain to be established.\(^{2–4}\) Early studies have shown that Lp(a) plays a potential role in thrombogenesis, interfering with several steps in the fibrinolytic pathway.\(^{5}\) Over the last years, new biological functions have been increasingly assigned to Lp(a), including activation of various cell types that play important roles in atherogenesis.\(^{6–9}\)

The biological effects of Lp(a) have been attributed either to apo(a) or to constituents of its LDL-like particle. Among the components of the LDL portion of Lp(a) is the enzyme platelet-activating factor acetylhydrolase. Platelet-activating factor acetylhydrolase primarily exhibits a Ca\(^{2+}\)-independent phospholipase A\(_{2}\) activity and is complexed to lipoproteins in plasma; thus, it is also referred to as lipoprotein-associated phospholipase A\(_{2}\) (Lp-PLA\(_{2}\)).\(^{10}\) Substrates for Lp-PLA\(_{2}\) are the proinflammatory phospholipid platelet activating factor, as well as phospholipids containing oxidatively fragmented residues at the sn-2 position (oxidized phospholipids; OxPLs).\(^{10}\) Such phospholipids are thought to play key roles in inflammatory reactions and particularly in vascular inflammation and atherosclerosis.\(^{11,12}\) The present review article focuses particularly on the characteristics of the Lp(a)-associated Lp-PLA\(_{2}\) and discusses the possible role of this enzyme in view of the new data showing that OxPLs in plasma are preferentially sequestered on Lp(a) and may significantly contribute to the atherogenicity of this lipoprotein.

Properties of the Lp(a)-Associated Lp-PLA\(_{2}\)

In normolipidemic human plasma with no detectable Lp(a) levels, Lp-PLA\(_{2}\) is mainly associated with LDL, whereas a
small proportion of enzyme activity is associated with high-density lipoprotein. According to previously published results by our group and others, LP-PLA₂ is also associated with LP(a). Interestingly, it was demonstrated that LP(a) is enriched in LP-PLA₂ because it contains a 1.5- to 2-fold higher enzyme mass and several-fold greater LP-PLA₂ activity compared with LDL when assayed at equimolar protein concentrations. This observation is likely to be important in understanding the role of both LP-PLA₂ and LP(a) in physiological conditions and in atherogenesis, in view of the observation that the enzyme substrates, OxPLs, in plasma are strongly associated with LP(a). The distribution of LP-PLA₂ between LDL and high-density lipoprotein in normocholesterolemic plasma can be influenced by the presence of LP(a) only when the plasma levels of LP(a) exceed 30 mg/dL. The LP(a)-associated LP-PLA₂ protein is highly glycosylated and has similar physicochemical and catalytic properties to those of LDL-associated LP-PLA₂. Furthermore, the enzyme activity on LP(a) or on LDL is susceptible to oxidation in vitro. The major role in the attachment of LP-PLA₂ on the LP(a) particles is played by the apolipoprotein B (apoB)-100 moiety of LP(a), whereas the enzyme does not bind to apo(a). Importantly, there are marked differences in the enzyme catalytic properties among the various LP(a) isoforms, the small isoforms exhibiting higher apparent Michaelis constant and maximum velocity values, compared with large ones, suggesting that the apo(a) may influence the association of LP-PLA₂ with LP(a), although it does not bind the enzyme itself.

Role of LP(a) and LP-PLA₂ in Atherogenesis
LP(a) is an atherogenic lipoprotein and it is present in atherosclerotic but not in normal vessel walls. In the early plaque, most of the LP(a) is located within endothelial cells where it significantly influences the cell function (reviewed in Reference 1). In advanced lesions, LP(a) is found predominantly in the intima, where it is primarily colocalized with foam cells. LP(a) contributes to the formation of foam cells because it can be oxidized, aggregated, or subjected to phospholipase A₂ modification, and in this form it is taken up by scavenger receptors of macrophages. In addition, LP(a) influences the function of cells that play important roles in atherogenesis, such as monocytes-macrophages and endothelial cells.

Unlike LP(a), the role of LP-PLA₂ in atherogenesis is less well understood. Results from in vitro experiments, as well as animal and human epidemiological studies, suggest that LP-PLA₂ may be atherothrombotic by documenting reduced oxidative stress, extent of atherosclerosis, and lower risk of myocardial infarction, stroke, and peripheral arterial disease. One caveat in these studies is that LP-PLA₂ may be mostly carried on high-density lipoprotein in the animal models studied; thus, the relevance to human disease is less certain, whereas LP-PLA₂ is present mostly on atherogenic LDL. Furthermore, the epidemiological data are primarily from Japanese populations carrying the V279F variant of the gene encoding LP-PLA₂. This population is characterized by the absence of circulating LP-PLA₂. More recent data show that the monocyte-derived macrophages of these individuals fail to secrete any LP-PLA₂, suggesting that this mutant impairs function of the LP-PLA₂ gene. Pathological data in white populations suggest that LP-PLA₂ may be proatherogenic, because it is present within atherosclerotic plaques and colocalizes with macrophages. In advanced lesions, LP-PLA₂ staining is intense in regions abundant in lipids and oxidation products. LP-PLA₂ is also present in thin-cap fibroatheromas, in necrotic cores of human ruptured plaques, and in apoptotic macrophages. This suggests that it is at least associated with plaque progression and vulnerability, although its exact role is not fully defined.

Consistent with the proatherogenic role of LP-PLA₂ are the results from large white population studies, which have demonstrated an independent association between plasma LP-PLA₂ mass and cardiovascular disease risk. In this regard, a recent meta-analysis showed that LP-PLA₂ is significantly associated with cardiovascular disease, and the risk estimate seems to be relatively unaffected by adjustment for conventional cardiovascular disease risk factors. Clinical studies are underway to assess the efficacy of specific inhibitors of LP-PLA₂, which, in animal studies, may confer protection against atherosclerosis.

LP(a)-Associated LP-PLA₂ in Atherosclerosis and Cardiovascular Disease
Despite the small contribution of the LP(a)-associated LP-PLA₂ in the plasma enzyme pool, the overall contribution of this enzyme in the inflammatory processes in the artery wall could be significant, especially in patients exhibiting high levels of LP(a) in plasma, because LP(a) accumulates preferentially to LDL within lesions, and much of it is very tightly bound to lesion components. Like LDL, LP(a) is susceptible to oxidation, and oxidized LP(a) is enriched in lysophosphatidylcholine (lyso-PC), which is formed by hydrolysis of OxPLs, a reaction catalyzed by the endogenous LP-PLA₂. Lyso-PC plays important roles in plaque formation; thus, it is considered as an important proatherogenic phospholipid. Thus, by mediating the hydrolysis of OxPL and the generation of lyso-PC, the LP(a)-associated LP-PLA₂ may significantly influence the biological activities of oxidized LP(a) in the artery wall, which are mediated by both OxPL and lyso-PC. A similar role has been described for the LDL-associated LP-PLA₂.

We had shown previously that the LP(a), which was isolated from plasma of patients with coronary artery disease (CAD), carries a significantly smaller amount of LP-PLA₂ mass, which expresses lower catalytic efficiency compared with controls, a phenomenon that is not observed for the LDL-associated LP-PLA₂. Importantly, the removal of apo(a) from the LP(a) particle resulted in a significant increase in the LP-PLA₂ catalytic efficiency, suggesting that the apo(a) moiety diminishes the enzyme activity expressed by LP(a) in CAD patients. Based on results from in vitro experiments, we may hypothesize that a major factor responsible for the low catalytic efficiency of the LP(a)-associated LP-PLA₂ in CAD patients may be the sequestration of OxPL on the apo(a) moiety of LP(a). These OxPLs could competitively inhibit the enzyme activity as it is determined in the...
Figure 1. Association of Lp-PLA₂ and OxPL with Lp(a) in normal plasma as well as in plasma of patients with CAD. Lp(a) of CAD patients contains higher levels of OxPL present on individual apoB-100 particles (OxPL/apoB levels), measured by the murine monoclonal antibody E06, are strongly correlated with plasma Lp(a) levels and are preferentially bound by Lp(a) compared with other apoB-containing lipoproteins. Furthermore, plasma OxPL/apoB levels are correlated with angiographically determined CAD and are elevated after acute coronary syndromes and immediately after percutaneous coronary intervention. In fact, in most conditions studied thus far, >90% of circulating OxPLs associated with apoB-100 particles are actually present on Lp(a). The only exception to this has been documented in patients undergoing percutaneous coronary intervention. Before percutaneous coronary intervention, most OxPLs in plasma were associated with Lp(a). Immediately after percutaneous coronary intervention, an acute increase was noted in plasma in both OxPL/apoB and Lp(a). However, only 50% of OxPLs were associated with Lp(a), whereas the remaining OxPLs were present on other non-Lp(a) apoB-100 particles. However, by 6 hours, >90% of the OxPLs were again present on Lp(a). This observation strongly supports a physiological function of Lp(a) in preferentially binding OxPLs compared with other apoB-100-containing particles and also suggests a transfer of OxPLs to Lp(a). More recently it was shown that OxPLs present primarily on Lp(a) are significant predictors of the presence and extent of carotid and femoral atherosclerosis, development of new lesions, and increased risk of cardiovascular events. Interestingly, this study further showed that the association of OxPL/apoB and Lp(a) with carotid and femoral atherosclerosis was strongest in subjects with the highest Lp(a) concentration but smallest apo(a) isoforms. Furthermore, the strongest correlation between OxPL/apoB and Lp(a) was noted in subjects with the smallest apo(a) isoforms, whereas those with higher numbers of isoforms (kringle IV type 2 repeats) had lower correlations (Spearman correlation between OxPL/apoB and apo(a) isoform numbers: ≥29 repeats, r=0.66; 23 to 29 repeats, r=0.88 and ≤22 repeats, r=0.93; P<0.001 each; Figure 2). According to our previously published results, one of the factors that could favor the sequestration of OxPL on small Lp(a) isoforms could be the low catalytic efficiency of the Lp-PLA₂ associated with these isoforms. The stronger association of OxPL with small Lp(a) isoforms may at least partially explain the enhanced presence of exogenously added substrate in the in vitro enzyme assays, thus leading to the expression of low catalytic efficiency by the Lp(a)-associated Lp-PLA₂.

Previous studies have demonstrated that OxPL can be directly bound to apo(a) forming covalent bonds with the active lysines of its kringle-V domain, although it is possible that there may be additional or alternative binding sites for OxPL on other kringles of apo(a). Furthermore, preliminary data suggest that additional OxPLs are present in the lipid phase of Lp(a) (Figure 1). These results may be influenced by both the heterogeneity of Lp(a), as well as the procedures used in isolating Lp(a) (ie, affinity chromatography using lysine-Sepharose versus other methods). An important role in the accumulation of OxPL on Lp(a) may be also played by β-2 glycoprotein I (β₂GPI), which binds to the kringle IV domain of apo(a), as well as to anionic phospholipids and OxPL. Importantly, we had shown previously that the α₂GPI levels on the Lp(a) of CAD patients are significantly higher compared with controls, whereas removal of apo(a) from the Lp(a) particles of these patients led to reduction of the α₂GPI levels and to increase in the Lp-PLA₂ catalytic efficiency. However, we may suggest that the higher amounts of α₂GPI on the Lp(a) of CAD patients could contribute to the sequestration of OxPL on the surface of Lp(a).

In addition to the low catalytic efficiency of Lp-PLA₂, the enzyme mass on Lp(a) of CAD patients is significantly reduced (Figure 1). The mechanism for this reduction is still unknown, because no data exist on the factors that could influence the binding of the enzyme on Lp(a), as well as how and where (in plasma, extracellularly, or intracellularly) the assembly between Lp-PLA₂ and Lp(a) occurs in vivo. Unpublished results from our laboratory have shown that the enrichment of Lp(a) in OxPL hinders the association of Lp-PLA₂ with Lp(a) in vitro. Consequently, we may suggest that the sequestration of OxPL on the surface of Lp(a) may account at least partially for the lower enzyme mass on Lp(a) of CAD patients, a hypothesis that needs further investigation.

Relationship of Oxidized Phospholipids With Lp(a)
The suggestion drawn from our previous results concerning the accumulation of OxPL on the Lp(a) of CAD patients is further supported by more recent data showing that plasma levels of OxPL present on individual apoB-100 particles (OxPL/apoB levels), measured by the murine monoclonal antibody E06, are strongly correlated with plasma Lp(a) levels and are preferentially bound by Lp(a) compared with other apoB-containing lipoproteins. Furthermore, plasma OxPL/apoB levels are correlated with angiographically determined CAD and are elevated after acute coronary syndromes and immediately after percutaneous coronary intervention. In fact, in most conditions studied thus far, >90% of circulating OxPLs associated with apoB-100 particles are actually present on Lp(a). The only exception to this has been documented in patients undergoing percutaneous coronary intervention. Before percutaneous coronary intervention, most OxPLs in plasma were associated with Lp(a). Immediately after percutaneous coronary intervention, an acute increase was noted in plasma in both OxPL/apoB and Lp(a). However, only 50% of OxPLs were associated with Lp(a), whereas the remaining OxPLs were present on other non-Lp(a) apoB-100 particles. However, by 6 hours, >90% of the OxPLs were again present on Lp(a). This observation strongly supports a physiological function of Lp(a) in preferentially binding OxPLs compared with other apoB-100-containing particles and also suggests a transfer of OxPLs to Lp(a). More recently it was shown that OxPLs present primarily on Lp(a) are significant predictors of the presence and extent of carotid and femoral atherosclerosis, development of new lesions, and increased risk of cardiovascular events. Interestingly, this study further showed that the association of OxPL/apoB and Lp(a) with carotid and femoral atherosclerosis was strongest in subjects with the highest Lp(a) concentration but smallest apo(a) isoforms. Furthermore, the strongest correlation between OxPL/apoB and Lp(a) was noted in subjects with the smallest apo(a) isoforms, whereas those with higher numbers of isoforms (kringle IV type 2 repeats) had lower correlations (Spearman correlation between OxPL/apoB and apo(a) isoform numbers: ≥29 repeats, r=0.66; 23 to 29 repeats, r=0.88 and ≤22 repeats, r=0.93; P<0.001 each; Figure 2). According to our previously published results, one of the factors that could favor the sequestration of OxPL on small Lp(a) isoforms could be the low catalytic efficiency of the Lp-PLA₂ associated with these isoforms. The stronger association of OxPL with small Lp(a) isoforms may at least partially explain the enhanced
atherogenicity of these isoforms as compared with large ones. In this regard it has been reported that high Lp(a) concentrations and low-molecular weight apo(a) isoforms are associated with preclinical vascular changes, cardiovascular disease, and the mode of presentation of CAD (acute coronary syndromes).42

The above observations suggest that the strong association and binding of OxPL by Lp(a) may be part of an innate immune response to detoxify proinflammatory OxPL released into the circulation from sources of cellular injury (such as plaque disruption and myocyte death) or apoptotic cells. These OxPLs are preferentially transferred to and sequestered by Lp(a) and are, thus, subjected to degradation catalyzed by the Lp(a)-associated Lp-PLA2 (Figure 3). This concept implies that, under normal conditions, some minimal levels of Lp(a) in the circulation may be beneficial by acting as a transporter and scavenger of OxPL. Under these conditions, the proinflammatory lyso-PC, which derives from the degradation of OxPL catalyzed by Lp-PLA2, could then be transferred from Lp(a) to albumin, which represents the major carrier of lyso-PC in plasma.43 However, under conditions of acute inflammation and oxidative stress, the Lp(a)-associated Lp-PLA2 activity may become insufficient to respond to the increased sequestration of OxPL on Lp(a). Under these conditions and especially in cases of increased Lp(a) plasma levels, this lipoprotein may become proatherogenic, particularly because of its enhanced binding to the vessel wall matrix. In the artery wall, Lp(a) will be accompanied by OxPL, the levels of which will be further increased because of the in situ Lp(a) oxidation, thus resulting in enhanced inflammation and progression of atherosclerosis.

Conclusions and Perspectives

It is now well established that Lp-PLA2 is intimately associated with Lp(a). Moreover, accumulated data show a strong association and binding of OxPL by Lp(a) in human plasma, thus providing evidence for an important role of Lp(a) as a scavenger of OxPL, which may be then potentially degraded by Lp(a)-associated Lp-PLA2. Lp(a) may significantly contribute or even primarily account for the atherogenicity of

Figure 2. 3D plot of OxPL/apoB levels according to Lp(a) mass and apo(a) phenotypes expressed as the number of kringle IV-type 2 repeats. OxPL/apoB levels are presented are geometric means (taken as the antiloge of the mean of log-transformed OxPL/apoB values). Reproduced, with permission, from Tsimikas S et al.41

Figure 3. Scavenging (binding) of OxPL by Lp(a) and detoxification (degradation) of OxPL by Lp(a)-associated Lp-PLA2. A, OxPLs are formed on oxidized LDL (OxLDL), as well as on apoptotic cells. OxPLs released into the circulation are preferentially transferred to Lp(a), the main acceptor of OxPL in plasma. The pathways and molecules associated with binding of OxPL to Lp(a) are not fully determined yet but may involve covalent binding to apo(a), binding to α2GPI, which attaches to Lp(a), and distribution in the lipid phase of Lp(a). B, Once OxPLs are bound on Lp(a), they become accessible to endogenous Lp-PLA2 and are degraded into lysophosphatidylcholine (lysoPC) and oxidized free fatty acids (OxFFA).
Lp(a) and its increased risk for cardiovascular disease. In addition, abnormal function of Lp(a)-associated Lp-PLA2, through several potential mechanisms as noted above, may predispose to pathways leading to atherogenesis and plaque rupture. Further studies will be required to assess the relationship between Lp-PLA2 mass and activity with OxPL and Lp(a) in large data sets. In addition, studies evaluating the coordinated role of OxPL and Lp-PLA2 on isolated Lp(a) will be invaluable to delineate the role of Lp(a)-associated Lp-PLA2 and to assess its pathophysiological and clinical relevance.

Sources of Funding
This work was supported by grants from the Greek Ministry of Research and Technology (PENED 2001 program), the Donald W. Reynolds Foundation, and the Fondation Leducq.

Disclosures
None.

References


New Insights Into the Role of Lipoprotein(a)-Associated Lipoprotein-Associated Phospholipase A2 in Atherosclerosis and Cardiovascular Disease
Sotirios Tsimikas, Loukas D. Tsironis and Alexandros D. Tselepis

Arterioscler Thromb Vasc Biol. 2007;27:2094-2099; originally published online July 12, 2007; doi: 10.1161/01.ATV.0000280571.28102.d4

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/10/2094

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/