Lipoprotein-associated Phospholipase A2: Current Trends in Invitro Diagnostics

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Abstract

Lipoprotein-associated phospholipase A2 is an imminent reliable precise biomarker for vascular inflammation involved in the development of unstable plaques in cardiovascular diseases. The atherosclerotic plaque formation, inflammation and rupture of arterial vessels lead to heart attacks and strokes in most of the cases. The vessel-specific inflammatory enzyme, Lipoprotein-associated phospholipase A2 shoot ups in response to the rupture of arterial vessels having atherosclerotic plaques. The accurate measurement of Lipoprotein-associated phospholipase A2 envisages the individual risk of a patient and the treatment can be customized based on the result. The measurement of inflammatory Lipoprotein-associated phospholipase A2 in non-cardiovascular diseases may be considered to identify patients at increased risk of stroke. The current strategy of hsCRP quantification in diagnosis of cardiovascular diseases can be beneficially substituted by Lipoprotein-associated phospholipase A2 as its expression is not affected by other bacterial or viral infections. This review will discuss about the significance of Lipoprotein-associated phospholipase A2 as a diagnostic marker and about the established diagnostics methods of the enzyme.

Keywords: Lipoprotein-associated phospholipase A2, Cardiovascular diseases. Biomarker, ELISA, Mass Spectrometry

Introduction

The Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a 45Kda hydrophobic protein encoded by PLA2G7 gene belongs to group VII of phospholipases A2 superfamily [1]. The Lp-PLA2 is circulating in blood in association to low density lipoprotein (LDL) by 70-80% and to high density lipoprotein (HDL) by 20-30% [2-5]. The Lp-PLA2 enzyme is produced mainly by intraplaque macrophages and foam cells and is released by plaque into the circulation, where it binds to lipoproteins [6]. The LP-PLA2 mainly functions by hydrolysing the phospholipids to oxidized fatty acids and lysophosphatidylcholine. These products will induce adhesion molecule expression and release of cytokines, which are involved in the formation of vascular inflammation and atherosclerosis [7]. Because of the hydrolysing activity of Lp-PLA2, the enzyme was first named as platelet-activating factor acetyl hydrolase (PAF-AH) and is also known as PLA2G7 enzyme, as it is encoded by the PLA2G7 gene [8]. The Lp-PLA2 is synthesized and secreted mainly by macrophages and by hematopoietic cells like monocytes, lymphocytes, mastocytes, platelets and hepatic cells.

The Lp-PLA2 is recently recognized as a non-traditional risk factor for major atherosclerotic cardiovascular disease (ASCVD) and is recommended to screen for dyslipidaemia [9]. Recent studies have been revealed the relevance of measurement of Lp-PLA2, that it is more specific than the high sensitive CRP (hsCRP). It will be useful in diagnosing the ASCVD risk in case of elevated hsCRP in other inflammatory conditions [10]. Lp-PLA 2 is also indorsed to predict ASCVD in individuals with low LDL cholesterol. The Lp-PLA2 is also listed under additional tests and is reported that the elevated Lp-PLA2 (≥200 ng/mL) has been independently linked with coronary events. Additionally, Lp-PLA2 may act in combination with CRP, further increasing risk when both are elevated [11]. It is also reported that the Lp-PLA2 measurement is useful in diagnosing of CVD in diabetes mellitus patients. The higher level of small dense LDL in diabetic patients make better transportation of Lp-PLA2 in circulation [12].

Lp-PLA2: Diagnostic Methodologies

The level of Lp-PLA2 in blood is normally measured either by colorimetric method or by immunometric method [13-17]. Mass Spectrometric based methods are also using for the direct quantification of the Lp-PLA2 protein [18]. The

colorimetric method expressed the enzyme activity and immunometric method provided the result in terms of concentration. Even though both the concentration and activity measurement are clinically useful, the comparison between these methods often causes discordance in result interpretation [19,20]. The currently available invitro diagnostics techniques for Lp-PLA2 detection are Enzyme Linked Immunosorbent Assay (ELISA), Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS), Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) immunoaffinity assay and colorimetric assay [21]. However, Lp-PLA2 concentration measurement has been regularly replaced, as it is less accurate than enzymatic activity assessment for risk stratification.

Chemical Method

A substrate possessing a colorimetric moiety can be used to measure Lp-PLA2 activity. Myristoyle-2-(p-nitrophenyl succinyl)-phosphatidylcholine, a Platelet activating factor (PAF) analogue with a 4- nitrophenyl group conjugated onto a succinyl chain at sn-2 position is used as the substrate [16,17]. Lp-PLA2 is a known hydrolyser of phospholipids. The PAF has a two-carbon acyl group at the sn-2 position; therefore, when PAF is hydrolysed by Lp-PLA2, the short acyl group is cleaved as water soluble acetate from the remainder of the molecule, which is Lyso-Phosphatidylcholine [22]. In the colorimetric test for Lp-PLA2 activity, Lp-PLA2 in plasma or serum, hydrolyses the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, producing 4-nitrophenol. The rate of formation of 4-nitrophenol is measured spectrophotometrically at 405 nm and the Lp-PLA2 activity is calculated from the rate of change in absorbance (fig. 1).

Enzymatic Method

The quantification of Lp-PLA2 can be done by enzymatic Lp-PLA$_2$ activity assay method. 1-*O*-Hexadecyl-2-acetyl-*rac*-glycero-3-phosphocholine (rac C_{16} PAF), a PAF analogue, is used as the substrate in enzymatic method. The substrate specificity of lysoplasmalogen-specific phospholipase D (lysophospholipase D (LysoPLD)) was exploited in this method. Lp-PLA$_2$ hydrolyses 1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (C_{16} PAF) to 1-*O*-Hexadecyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPAF). Then the LysoPLD acted on LysoPAF, and the hydrolytically release choline which will be detected by the choline oxidase (fig. 2) The reference value for Lp-PLA2 in enzymatic method is up to 40U/L and the measuring range of the assay is 6-120 U/L.

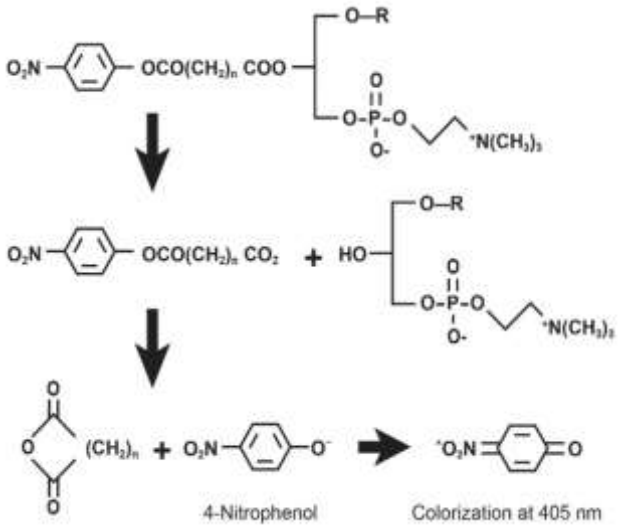

Fig.1; Lp-PLA2 Colorimetric Reaction

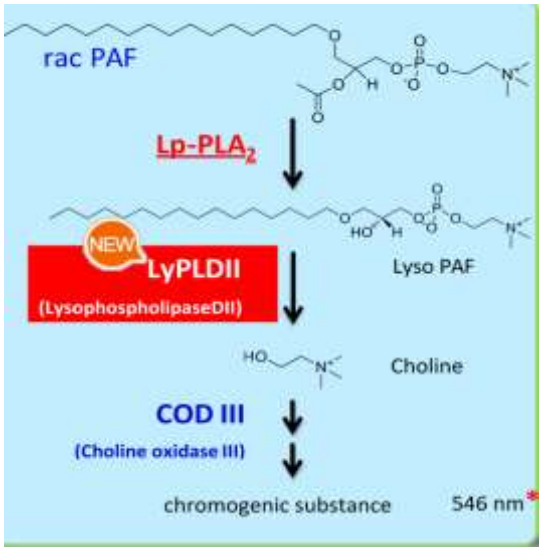

Fig.2; Lp-PLA2 Enzymatic Reaction

ELISA for Lp-PLA2

The mass assay or concentration assay for Lp-PLA2 is performed by solid phase sandwich immunoassay. Two different monoclonal antibodies having far distantly placed epitopes for Lp-PLA2 is used in the assay for binding with the Lp-PLA2 enzyme antigen. The horse radish peroxidase (HRP) conjugated secondary antibody will impart the colour reaction upon react with the tetramethylbenzidine substrate. Upon stopping the reaction with 1N H2SO4, the absorbance of the enzymatic turnover of the substrate is determined spectrophotometrically at 450 nm and is directly proportional to the concentration of Lp-PLA2. A set of Lp-PLA2 Calibrators is used to plot a standard curve of absorbance versus Lp-PLA2 concentration from which the Lp-PLA2 concentration in the test sample can be determined. It is reported that the use of non-ionic surfactants in sample diluting buffer enhance the sensitivity of Lp-PlA2 assay. As the Lp-PLA2 is associated with lipoproteins, the treatment with detergents liberates the protein from lipoprotein complex and thereby increase the detection level [23].

After the first FDA approved kit for Lp-PLA2 from the manufacturer Diadexus, ELISA based kits for Lp-PLA2 concentration quantification have been marketed by several renowned invitro diagnostics companies [24]. Normally the assay range for Lp-PLA2 in ELISA kits ranges from 8ng/ml to 2000ng/ml. The assay result of above 200ng/mL in ELISA is considered as high CVD risk.

LC-MS/MS for Lp-PLA2

The LC-MS/MS technology enables the accurate, separate identification and quantification of the Lp-PLA2 without the interference of closely associated metabolites and non-protein moieties. The substrate used in mass spectrometry for Lp-PLA2 assay is the deuterated PAF C-16 and serially diluted (50umol/L to 0.5umol/L) LysoPAF C-16 is used as the calibrator. The lysophosphatidyl choline is employed as the internal standard. Based on the calibration curve and the internal standard peak the analyte concentration can be assessed in the unknown sample. Even though the mass spectrometry assays are superior in Lp-PLA2 quantification than other immunoassays, the high cost of equipment and need of trained technical expertise makes the techniques less applicable for diagnostics [25].

SISCAPA for Lp-PLA2

The SISCAPA immunoaffinity assay is an improved Mass Spectrometry platform which combines the MS specificity with the immunoassay sensitivity. It is a directed assay methodology for sensitive, precise and high throughput measurement of small peptides and metabolites. In this method, synthetic stable isotope labelled peptides having structural similarity with the target peptide is used as the internal standard. For Lp-PLA2 assay, the ^{15}N-labelled Lp-PLA2 can be used as the internal standard. Serially diluted recombinant Lp-PLA2 (2000ng/mL to 100ng/mL) can be used as the calibrators. The samples for analysis will be subjected to trypsin proteolysis for the digestion of target protein. The Lp-PLA2 anti-peptide monoclonal antibody conjugated with magnetic bead or immobilized on affinity column facilitates the binding of proteolyzed peptides to the specific antibody molecules. After 2 hours of incubation the unbound peptides were washed out by phosphate buffered saline – CHAPS buffer and elute the peptides from bound antibodies by an acidic buffer. Then the eluted target peptides will be subjected to quantitative mass spectrometry. The SISCAPA immunoaffinity assay offers better sensitivity than the LC-MS/MS for Lp-PLA2 quantification [21].

Conclusion

Quantitative measurements of Lp-PLA2 serves as an independent specific risk predictor of cardiovascular disease helps the clinicians to provide effective treatment to those high-risk individuals. High-sensitive CRP is also a predictor of cardiovascular disease, but higher levels are seen in inflammation and infections. The hsCRP also depends on other factors such as age, smoking and obesity. The hsCRP measurements during tissue injuries, infections and general inflammations can lead to false interpretations on cardiovascular disease. Considering these variations and non-specificity of hsCRP, more specific Lp-PLA2 estimations play a pivotal role in cardiovascular health management. Further innovations are needed in invitro diagnostics to improve the analytical sensitivity and accuracy of Lp-PLA2 assay with an affordable cost.

Compliance with Ethical Standards

Conflict of interest; All the authors declare that there is no conflict of interest.

References

1. Stafforini, D., 2008. Biology of Platelet-activating Factor Acetylhydrolase (PAF-AH, Lipoprotein Associated Phospholipase A2). Cardiovascular Drugs and Therapy, 23(1), pp.73-83.
2. Karasawa K, Inoue K. Overview of PAF-Degrading Enzymes. Enzymes. 2015; 38:1-22. doi: 10.1016/bs.enz.2015.09.006. Epub 2015 Nov 6. PMID: 26612643.
3. Palur Ramakrishnan, A., Varghese, T., Vanapalli, S., Nair, N. and Mingate, M., 2016. Platelet activating factor: A potential biomarker in acute coronary syndrome? Cardiovascular Therapeutics, 35(1), pp.64-70.
4. Stafforini, D., McIntyre, T., Carter, M. and Prescott, S., 1987. Human plasma platelet-activating factor acetylhydrolase. Association with lipoprotein particles and role in the degradation of platelet-activating factor. Journal of Biological Chemistry, 262(9), pp.4215-4222.
5. Stafforini, D., Prescott, S. and McIntyre, T., 1987. Human plasma platelet-activating factor acetylhydrolase. Purification and properties. Journal of Biological Chemistry, 262(9), pp.4223-4230.
6. Elstad, M., Stafforini, D., McIntyre, T., Prescott, S. and Zimmerman, G., 1989. Platelet-activating Factor Acetylhydrolase Increases during Macrophage Differentiation. Journal of Biological Chemistry, 264(15), pp.8467-8470.
7. Biasucci, L., Vitelli, A., Liuzzo, G., Altamura, S., Caligiuri, G., Monaco, C., Rebuzzi, A., Ciliberto, G. and Maseri, A., 1996. Elevated Levels of Interleukin-6 in Unstable Angina. Circulation, 94(5), pp.874-877.
8. Tjoelker, L., Wilder, C., Eberhardt, C., Stafforinit, D., Dietsch, G., Schimpf, B., Hooper, S., Trong, H., Cousens, L., Zimmerman, G., Yamadat, Y., Mclntyre, T., Prescott, S. and Gray, P., 1995. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature, 374(6522), pp.549-553.
9. Alberti, K., Eckel, R., Grundy, S., Zimmet, P., Cleeman, J., Donato, K., Fruchart, J., James, W., Loria, C. and Smith, S., 2009. Harmonizing the Metabolic Syndrome. Circulation, 120(16), pp.1640-1645.
10. Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P. and Garg, R., 2005. Metabolic Syndrome. Circulation, 111(11), pp.1448-1454.
11. Libby P., Ridker P. M., Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135–1143. doi: 10.1161/hc0902.104353.

12. Eckel, R., Wassef, M., Chait, A., Sobel, B., Barrett, E., King, G., Lopes-Virella, M., Reusch, J., Ruderman, N., Steiner, G. and Vlassara, H., 2002. Prevention Conference VI: Diabetes and Cardiovascular Disease. Circulation, 105(18).
13. AARSMAN, A., NEYS, F. and BOSCH, H., 1991. Catabolism of platelet-activating factor and its acyl analog. Differentiation of the activities of lysophospholipase and platelet-activating-factor acetylhydrolase. European Journal of Biochemistry, 200(1), pp.187-193.
14. Caslake, M., Packard, C., Suckling, K., Holmes, S., Chamberlain, P. and Macphee, C., 2000. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. Atherosclerosis, 150(2), pp.413-419.
15. Petrovic, N., Grove, C., Langton, P., Misso, N. and Thompson, P., 2001. A simple assay for a human serum phospholipase A2 that is associated with high-density lipoproteins. Journal of Lipid Research, 42(10), pp.1706-1713.
16. Donato, L., Meeusen, J., Callanan, H., Saenger, A. and Jaffe, A., 2016. Advantages of the lipoprotein-associated phospholipase A2 activity assay. Clinical Biochemistry, 49(1-2), pp.172-175.
17. Kosaka, T., Yamaguchi, M., Soda, Y., Kishimoto, T., Tago, A., Toyosato, M. and Mizuno, K., 2000. Spectrophotometric assay for serum platelet-activating factor acetylhydrolase activity. Clinica Chimica Acta, 296(1-2), pp.151-161.
18. De Stefano, A., Mannucci, L., Tamburi, F., Cardillo, C., Schinzari, F., Rovella, V., Nisticò, S., Bennardo, L., Di Daniele, N. and Tesauro, M., 2019. Lp-PLA2, a new biomarker of vascular disorders in metabolic diseases. *International Journal of Immunopathology and Pharmacology*, 33, p.205873841982715.
19. Persson, M., Hedblad, B., Nelson, J. and Berglund, G., 2007. Elevated Lp-PLA 2 Levels Add Prognostic Information to the Metabolic Syndrome on Incidence of Cardiovascular Events Among Middle-Aged Nondiabetic Subjects. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(6), pp.1411-1416.
20. Zalewski, A., Nelson, J., Hegg, L. and Macphee, C., 2006. Lp-PLA2: A New Kid on the Block. Clinical Chemistry, 52(9), pp.1645-1650.
21. Topbas, C., Swick, A., Razavi, M., Anderson, N., Pearson, T. and Bystrom, C., 2018. Measurement of Lipoprotein-Associated Phospholipase A2 by Use of 3 Different Methods: Exploration of Discordance between ELISA and Activity Assays. Clinical Chemistry, 64(4), pp.697-704.
22. Stafforini, D., McIntyre, T., Zimmerman, G. and Prescott, S., 1997. Platelet-activating Factor Acetylhydrolases. Journal of Biological Chemistry, 272(29), pp.17895-17898.
23. Zhuo, S., Wolfert, R. and Yuan, C., 2017. Biochemical differences in the mass and activity tests of lipoprotein-associated phospholipase A 2 explain the discordance in results between the two assay methods. Clinical Biochemistry, 50(18), pp.1209-1215.
24. Mannheim, D., Herrmann, J., Versari, D., Gössl, M., Meyer, F., McConnell, J., Lerman, L. and Lerman, A., 2008. Enhanced Expression of Lp-PLA 2 and Lysophosphatidylcholine in Symptomatic Carotid Atherosclerotic Plaques. Stroke, 39(5), pp.1448-1455.