How does rupture of an atherosclerotic plaque activate platelets, and what can be done to lessen the risk of an ensuing thrombotic episode? Several important new observations that may provide answers to these vital questions are described by Rother et al. in the present issue of *Circulation*. This group previously reported that lysophosphatidic acid (LPA), a phospholipid that activates platelets, is contained in the lipid-rich core of human atherosclerotic lesions. They now identify the molecular species of LPA contained in the lipid-rich core of human atherosclerotic plaques. Mildly oxidized LDLs contain LPA, and some of the LPA in the plaque probably is produced in the core of carotid artery plaques obtained from surgical specimens, demonstrate that lipid extracted from soft plaques can activate human platelets, and show that LPA receptor antagonists will reduce the likelihood of thrombosis after plaque rupture. This is an interesting new approach that deserves careful consideration.

## Phospholipids and LPA

LPA is an intermediate in phospholipid metabolism that ordinarily is present in very small amounts in human plasma and tissues. It is a member of the lysophospholipid class of phospholipids. Lysophospholipids are formed by removal of one of the 2 fatty acid chains attached to the glycerol backbone of phosphoglycerides, the most abundant form of phospholipids. What makes LPA different from other lysophospholipids is that it does not have a head group, such as choline or ethanolamine, attached to its phosphate moiety. Many cells can produce LPA and, in addition, have LPA receptors. Therefore, LPA can act as either an autocrine or paracrine mediator, and it is an important intracellular messenger that can affect many diverse biological processes.

It is likely that 2 mechanisms account for the accumulation of LPA in the lipid core of atheromas. Mildly oxidized LDLs contain LPA, and some of the LPA in the plaque probably is directly deposited by the LDL that enters the arterial wall and undergoes oxidation. The rest probably is formed from the phosphoglycerides of macrophages and smooth muscle cells that are present in the developing atherosclerotic lesion. In theory, LPA can be produced from any phosphoglyceride, but quantitative and metabolic considerations suggest that the most likely source is the choline phosphoglycerides.

## LPA Formation

Two hydrolytic enzymes are necessary to convert phosphoglycerides to LPA. They are phospholipase A, which removes 1 of the 2 fatty acids from the glycerol backbone, and phospholipase D, which removes the head group from the phosphate. Atherosclerotic lesions contain phospholipase A (group II sPLA2), a form of phospholipase A that selectively removes the fatty acid present in the sn-2 position—the carbon of the glycerol backbone that is next to the one containing the phosphate group. Many cells have phospholipase D, including monocytes, endothelial cells, and aortic smooth muscle cells, so this enzymatic activity almost certainly also is expressed in atherosclerotic lesions.

Phospholipase A and phospholipase D can operate in 2 sequences. In one, the sn-2 fatty acid is removed first by phospholipase A, followed by removal of the head group from the resulting lysophospholipid by phospholipase D. This sequence occurs when LPA is formed by activated platelets. In the other sequence, phospholipase D acts first to form phosphatic acid, a phosphoglyceride that does not contain a head group, and the sn-2 fatty acid is removed from phosphatidic acid by phospholipase A. Many intracellular signaling pathways utilize this sequence to synthesize LPA.

Alternatively, LPA might be formed as a result of an abnormality in lipid synthesis. Phosphatic acid is an intermediate in the pathways for phosphoglyceride and triglyceride synthesis, and it could accumulate and be converted to LPA by phospholipase D if the final steps in these pathways were inoperative. Although this possibility cannot be excluded, it is much more likely that LPA is produced in the atheroma as a result of phospholipid degradation.

Most of the LPA found in the carotid artery plaques is acyl-LPA. The fatty acid in acyl-LPA is attached to the glycerol backbone in ester linkage, just as it usually is in phospholipids. However, 20% is alkyl-LPA, a form in which the fatty acid is attached to glycerol through an ether bond. Alkyl-LPAs are the most potent platelet-activating form of LPA. They are produced from alkyl-glycerophosphatides, a phosphoglyceride subclass in which one of the fatty acids is attached to glycerol through an ether bond. However, the sn-2 fatty acid has the usual ester bond, and phospholipase A can remove it. Therefore, alkyl-LPAs can be produced from alkyl-glycerophosphatides by the phospholipase A pathway.

What is difficult to reconcile with this enzymatic mechanism is the finding that a small amount of arachidonyl LPA is produced from double bond migration of lysophosphatidic acid (LPA) by phospholipase D.
present in the atheroma lipid. Arachidonic acid ordinarily is attached to the sn-2 position in phospholipids, and it should be removed by phospholipase A2 when a phosphoglyceride is converted to LPA. The position of the arachidonic acid on the glycerol backbone will have to be determined in order to provide some insight into how arachidonoyl LPA might be formed.

Oxidized LDL and LPA Production

The fact that LPA is present in mildly oxidized LDL suggests that there may be a connection between its formation and the oxidative processes involved in atherogenesis.5-7 Mildly oxidized LDL contains phosphoglycerides that have oxo-ovaleryl, glutaroyl, and epoxyisoprostanoyl groups instead of ordinary fatty acids in the sn-2 position.10 They are produced by oxidation of either arachidonic acid or another polyunsaturated fatty acid susceptible to peroxidation that originally was present in the sn-2 position of the phosphoglyceride. The resulting oxidized phospholipids are atherogenic.11 Platelet-activating factor acetylhydrolase, which is associated with LDL,7 can remove the sn-2 oxidized residues and thereby inactivate the oxidized phospholipids.10 The resulting lysophospholipids might then be converted to LPA when the LDL comes in contact with cells that contain phospholipase D. Thus, LPA formation in LDL may be a consequence of LDL oxidation.

LPA Receptors and Platelet Activation

There are 3 LPA receptor subtypes, LPA1, LPA2, and LPA3; they are also called endothelial differentiation gene (EDG)-2, EDG-4, and EDG-7, respectively.12 These are G protein–coupled receptors that affect many processes, including angiogenesis and wound healing, cardiac development, vascular smooth muscle activation, neuronal survival, and immunity.12,13 Each of the 3 LPA receptor subtypes are expressed in human platelets.14 The LPA-mediated signaling mechanism in platelets activates the GTPase RhO and Rh kinase.15 This leads to phosphorylation of myosin light chains and moesin, causing cytoskeletal rearrangement. The resulting shape change activates the platelet, sensitizing it to aggregation by subthreshold concentrations of adenosine diphosphate or epinephrine.1

LPA Receptor Antagonists

Dioctyglycerol pyrophosphate (DGPP) and dioctylphosphatic acid (PA-C14) are selective antagonists of LPA1 and LPA2.16 Unlike ordinary phospholipids that have fatty acids containing 14 to 22 carbons, these phosphatic acid analogues have 8-carbon fatty acid chains. DGPP and PA-C14 block LPA- and atheroma lipid–induced platelet activation.1 Therefore, the platelet response to LPA is mediated by either LPA1 or LPA3, or the combined action of the 2 receptors, but not by LPA2. DGPP is the more effective antagonist, it is specific for LPA-induced platelet activation, its effect is reversible, and it does not interfere with other platelet agonists. Consequently, DGPP may afford protection against thrombosis induced by plaque rupture without causing undue risk of bleeding. N-Palmityl-L-serine phosphoric acid and N-palmitoyl-L-tyrosine phosphoric also are potent, selective antagonists of LPA receptors, and they inhibit LPA-induced platelet activation.17,18 Like DGPP, these compounds may be prototypes for the development of a pharmacological agent designed to protect against thrombosis resulting from plaque rupture.

Will LPA Receptor Antagonists Reduce the Risk of Coronary Thrombosis?

LPA receptor antagonists unquestionably have potential as therapeutic agents, but caution should be exercised before committing to this approach for protection against coronary thrombosis. The present results were obtained entirely with lipid extracted from carotid plaques. Although it is reasonable to assume that LPA also is contained in other atheromas, it still is essential to show that lipid from coronary plaques produces LPA-dependent platelet activation before considering this approach for coronary disease. Furthermore, the lipid extracted from only 7 of the 12 carotid specimens tested consistently produced a platelet shape change. The reason for the inconsistency needs to be explored. Does the LPA-dependent mechanism operate in only a subset of atheromas, and if so, can these plaques be identified?

Finally, and perhaps most importantly, LPA is not the only thrombogenic lipid detected in atheromas. An alkyl glycerophosphatid containing an oxovaleryl residue in the sn-2 position, which is present in human aortic atheromas, aggregates rabbit platelets.19 Cholesterol 3-sulfate, which also is present in human aortic atheromas, increases platelet adhesion.20 LPA receptor blockade is unlikely to prevent thrombosis if platelets are exposed to either of these compounds when a plaque ruptures.

In summary, LPA receptor blockade is a promising new approach for reducing the risk of thrombosis associated with plaque rupture. However, a number of key issues must be resolved to determine its ultimate therapeutic usefulness.

Acknowledgments

The author is supported by research grant HL72845 and program project grants HL49264 and HL62984 from the National Heart, Lung, and Blood Institute, National Institutes of Health.

References


**Key Words:** Editorials ■ atherosclerosis ■ plaque ■ lipids ■ thrombosis
Plaque Rupture, Lysophosphatidic Acid, and Thrombosis
Arthur A. Spector

Circulation. 2003;108:641-643
doi: 10.1161/01.CIR.000082307.85449.1D
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/108/6/641

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation 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 Circulation is online at:
http://circ.ahajournals.org//subscriptions/