**Editorial**

**Plaque Rupture, Lysophosphatidic Acid, and Thrombosis**

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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 present 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 inhibit this process. Rother et al conclude that when a plaque ruptures, LPA contained in the exposed lipid sensitizes platelets to aggregating agents and thereby increases the thrombogenic potential. Furthermore, they suggest that LPA receptor antagonists will reduce the likelihood of thrombosis after plaque rupture. This is an interesting new approach that deserves careful consideration.

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 phospate. 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 phosphatic 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. Phosphatidic 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 alkylglycerophosphatides, 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--phospholipase D pathway.

What is difficult to reconcile with this enzymatic mechanism is the finding that a small amount of arachidyl LPA is...
present in the atheroma lipid.\(^1\) Arachidonic acid ordinarily is attached to the \(sn-2\) position in phospholipids, and it should be removed by phospholipase \(A_2\) 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.\(^4\)\(^-\)\(^5\) Mildly oxidized LDL contains phosphoglycerides that have oxo-ovaleryl, glutaroyl, and epoxyisoprostanoic acid 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 acetylhydrolyase, 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, LPA\(_1\), LPA\(_2\), and LPA\(_3\); 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 Rho 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.\(^3\)

### LPA Receptor Antagonists

Diocetylglycerol pyrophosphate (DGPP) and dioctylphosphatidic acid (PA-C\(_4\)) are selective antagonists of LPA\(_1\) and LPA\(_2\).\(^16\) Unlike ordinary phospholipids that have fatty acids containing 14 to 22 carbons, these phosphatidic acid analogues have 8-carbon fatty acid chains. DGPP and PA-C\(_4\) block LPA- and atheroma lipid–induced platelet activation.\(^1\) Therefore, the platelet response to LPA is mediated by either LPA\(_1\) or LPA\(_2\), or the combined action of the 2 receptors, but not by LPA\(_3\). 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-Palmitoyl-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 glycerophosphatidate containing an oxovaleryl residue in the \(sn-2\) position, which is present in human aortic atheromas, aggre gates 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.

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### References


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