Subtype-Selective Antagonists of Lysophosphatidic Acid Receptors Inhibit Platelet Activation Triggered by the Lipid Core of Atherosclerotic Plaques

Enno Rother, BS; Richard Brandl, MD; Daniel L. Baker, PhD; Pankaj Goyal, MSc; Harry Gebhard, BS; Gabor Tigyi, MD, PhD; Wolfgang Siess, MD

Background—Lysophosphatidic acid (LPA) is a platelet-activating component of mildly oxidized LDL (mox-LDL) and lipids isolated from human atherosclerotic plaques. Specific antagonists of platelet LPA receptors could be useful inhibitors of thrombus formation in patients with cardiovascular disease.

Methods and Results—Short-chain analogs of phosphatidic acid (PA) were examined for their effect on two initial platelet responses, platelet shape change and Ca$^{2+}$/H$^{+}$ mobilization. Dioctylglycerol pyrophosphate [DGPP(8:0)] and dioctylphosphatidic acid [PA(8:0)], recently described selective antagonists of the LPA$^1$ and LPA$^3$ receptors, inhibited platelet activation evoked by LPA but not by other platelet stimuli. DGPP(8:0) was more potent than PA(8:0). DGPP(8:0) also inhibited platelet shape change induced by mox-LDL and lipid extracts from human atherosclerotic plaques. Notably, we demonstrate for the first time that the lipid-rich core isolated from soft plaques was able to directly induce shape change. This effect was completely abrogated by prior incubation of platelets with DGPP(8:0). Moreover, coapplication of the lipid-rich core or LPA together with subthreshold concentrations of ADP or epinephrine synergistically induced platelet aggregation; this effect was inhibited by DGPP(8:0). Analysis by liquid chromatography-mass spectrometry revealed the presence of LPA alkyl- and acyl-molecular species with high platelet-activating potency (16:0-alkyl-LPA, 20:4-acyl-LPA).

Conclusions—LPA molecules present in the core region of atherosclerotic plaques trigger rapid platelet activation through the stimulation of LPA$^1$ and LPA$^3$ receptors. Antagonists of platelet LPA receptors might provide a new strategy to prevent thrombus formation in patients with cardiovascular diseases. (Circulation. 2003;108:741-747.)

Key Words: platelets receptor lipids plaque lipoproteins

Oxidative modification of LDL and platelet activation plays a central role in the pathogenesis of atherosclerosis and cardiovascular diseases.1 Lipid-rich atheromatous plaques containing oxidized lipids and LDL are vulnerable, and, on rupture, circulating platelets come into contact with this highly thrombogenic material.2 Indeed, oxidatively modified LDL such as mildly oxidized LDL (mox-LDL) and lipid extracts of human atherosclerotic plaques have been shown to stimulate platelets.3,4 Platelets once activated change their shape, aggregate, and become procoagulant, leading to the formation of a platelet- and fibrin-rich intravascular thrombus, which can precipitate acute ischemic syndromes such as unstable angina, myocardial infarction, and stroke. Oxidized LDL is also present in circulating blood and could be responsible for the enhanced platelet aggregability and the prothrombotic state often observed in patients with cardiovascular disease.5–7

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By studying the interaction of oxidatively modified LDL with platelets, we previously discovered that lysophosphatidic acid (LPA) is formed during mild oxidation of LDL and is responsible for platelet activation induced by mox-LDL and minimally modified LDL.4 Consistent with this observation, we also found that LPA accumulates in the intima of human atherosclerotic lesions.5 Along with LPA, other platelet agonists have been found in human atherosclerotic lesions.8–10 However, the relative importance of these potentially thrombogenic substances in mediating platelet activation by human atherosclerotic plaques is unknown. Until now, direct platelet activation by the plaque lipid core has not been reported.

LPA binds to and activates G-protein-coupled LPA receptors (GPCRs), including LPA$^1$, LPA$^2$, and LPA$^3$.11 All
three LPA receptors have been detected in platelets by RT-PCR. However, the LPA receptor(s) responsible for mediating LPA-induced platelet activation is not yet known. Activation of LPA receptors in various cell types leads to the stimulation of the heterotrimeric G-proteins G\(_{12/13}\), G\(_i\), and G\(_o\). It was previously found that LPA and mox-LDL activate Rho and Rho-kinase through G\(_{12/13}\) and that this pathway mediates the reorganization of the actin cytoskeleton underlying platelet shape change. In addition, LPA and mox-LDL stimulate a different pathway during shape change, that is, the activation of the Src family of tyrosine kinases and the tyrosine kinase Syk, which mediates the exposure of fibrinogen-binding sites on integrin \(\alpha IIb\beta 3\) during shape change, which is a prerequisite for platelet aggregation.

We have screened natural and synthetic analogs of LPA and PA in an effort to describe compounds that inhibit LPA-induced platelet responses. In this study, we report that dioctylglycerol pyrophosphate [DGPP(8:0)] and dioytylphosphatic acid [PA(8:0)] selectively inhibit platelet shape change induced by LPA and mox-LDL. DGPP(8:0) and PA(8:0) have been recently identified as antagonists of LPA\(_1\) and LPA\(_3\) receptors, but not of the LPA\(_2\)-receptor. Importantly, we demonstrate for the first time that the lipid-rich core of atheromatous plaques was able to directly trigger platelet activation. Platelet shape change and synergistic platelet aggregation induced by plaque lipid-rich core was completely inhibited by prior incubation of platelets with DGPP(8:0). These results indicate that LPA is an important thrombogenic component of the plaque lipid core, which mediates platelet activation through the stimulation of the LPA\(_1\) and LPA\(_3\) receptors.

Methods

Materials

DGPP(8:0), diacyl-(8:0)-glycerophosphate (phosphaticid acid [8:0]; PA[8:0]), and their long-chain analogs diacyl-(18:1)-glycerophosphate [DGPP(18:1)], diacyl-(18:1)-glycerophosphate (PA 18:1), diacyl-(18:0)-glycerophosphate (PA 18:0), were obtained from Avanti Polar Lipids. 1-acyl-(8:0)-glycerophosphate (lyosphosphaticid acid [8:0]; LPA(8:0)), 1-acyl-(20:4)-glycerophosphate (acyl-LPA 20:4), 1-acyl-(18:1)-sn-2, 3-cyclic glycerophosphate (cPA 18:1), 1-hexadecyl-LPA (alkyl-LPA 16:0), and N-palmitoyl-tyrosine phosphoric acid (NPTyrPA) were synthesized as described previously. 1-Oleoyl-LPA (LPA 18:1), ADP, U46619, human thrombin, 1-Palmitoyl-LPA (acyl-LPA 16:0), and their long-chain analogs diacyl-(18:1) phosphatidic acid [PA(18:1)], diacyl-(8:0)-glycerophosphate (phosphatidic acid [8:0]; PA(8:0)) have been recently identified as antagonists of LPA\(_1\) and LPA\(_3\) receptors, but not of the LPA\(_2\)-receptor. 20 Importantly, we demonstrate for the first time that the lipid-rich core of atheromatous plaques was able to directly trigger platelet activation. Platelet shape change and synergistic platelet aggregation induced by plaque lipid-rich core was completely inhibited by prior incubation of platelets with DGPP(8:0). These results indicate that LPA is an important thrombogenic component of the plaque lipid core, which mediates platelet activation through the stimulation of the LPA\(_1\) and LPA\(_3\) receptors.

Isolation of Platelets and Measurement of Platelet Shape Change and Aggregation

For measurement of shape change, human platelets were treated with acetylsalicylic acid and isolated in the presence of apyrase, as described previously. Platelets were resuspended at a concentration of 4\(\times\)10\(^5\)/mL in buffer C (20 mmol/L HEPES, 138 mmol/L NaCl, 2.9 mmol/L KCl, 1 mmol/L MgCl\(_2\), 0.36 mmol/L NaH\(_2\)PO\(_4\), 5 mm glucose; pH 7.4) containing either apyrase (0.6 U ADPase/mL) or CP (1 mmol/L)/CPK (10 U/mL) to remove extracellular ADP. The phospholipids (10 mmol/L, dissolved in methanol) were diluted 1:10 in buffer (20 mmol/L HEPES, 138 mmol/L NaCl, 0.25 mmol/L DGPP(8:0) and PA(8:0)) could also be directly resuspended in the albumin buffer at a concentration of 1 to 4 mmol/L. Suspensions of washed platelets were incubated at 37°C with various concentrations of the lipids or vehicle control before exposure to LPA, plaque material, or mox-LDL. Shape change was measured by the decrease in light transmission of the stirred (1100 rpm) platelet suspension in a LABOR aggrerometer (Fresenius).

For measurement of aggregation, human platelets were isolated according to methods previously described. After the first wash, platelets were resuspended in the second albumin-free wash solution (20 mmol/L HEPES, 138 mmol/L NaCl, 2.9 mmol/L KCl, 1 mmol/L MgCl\(_2\), 0.36 mmol/L NaH\(_2\)PO\(_4\), 5 mm glucose; pH 6.2), centrifuged, and resuspended in albumin-free buffer C. Fibrinogen (0.5 mg/mL) was added, and aggregation was measured by the increase of light transmission through the stirred platelet suspension in the aggrerometer.

Fluorescence Microscopy of F-Actin-Stained Platelets

Platelet shape change was also morphologically examined after phallolidin staining of F-actin as described. Fluorescence microscopy was performed using a Zeiss LSM510 META confocal fluorescence microscope and Zeiss software.

Measurement of Cytosolic Ca\(^{2+}\) Concentration

Measurement of cytosolic Ca\(^{2+}\) concentrations was done as previously described.

Isolation of Lipid-Rich Core From Human Carotid Atherosclerotic Plaques

Atherosclerotic tissue specimens were obtained from patients who underwent operations for high-grade carotid stenosis. Consent of the patients was obtained in a form approved by the Ethics Committee of the Faculty of Medicine of the Technical University of Munich. The carotid plaque tissue was removed by a technique of intraoperative endarterectomy that preserved the plaque structure en bloc. The specimens containing soft, lipid-rich plaques were collected. The lipid-rich core region was carefully dissected, weighed, and homogenized in ice-cold N\(_2\) saturated buffer containing NaCl (150 mmol/L) and EDTA (1 mmol/L), and stored at −20°C.

Lipid Extraction From the Plaque Lipid Core, Bioassay, and LC-MS Determination of LPA

Some of the samples containing plaque lipid-rich core material were extracted as described. The samples (0.5 mL, 50 mg/mL) were spiked either with [oleoyl-9,10-\(^3\)H]LPA (NEN) for determinations of recovery for quantification of LPA by bioassay or with 1 mmol/L deuterated LPA (LPA 18:0-d\(_{4}\)) for quantification of LPA by electrospray ionization liquid chromatography–mass spectrometry (ESI-LC-MS).

Preparation of Mox-LDL

The isolation of LDL from human plasma and its mild oxidation were carried out as described previously.

Results

DGPP(8:0) Lacks Agonist Activity and Specifically Inhibits Platelet Shape Change Induced by LPA

Low concentrations of acyl-LPA (18:1) with an EC\(_50\) of 7.4 mmol/L (mean±SD, n=22) and maximum of 50 to 100 mmol/L induce platelet shape change without stimulating secretion or aggregation. By studying several natural and synthetic analogs of LPA and PA for their effect on platelet shape change induced by LPA (Table), we found that the short-chain phosphatidates DGPP(8:0) and PA(8:0) inhibited LPA-induced shape change and were without agonist activity.
DGPP(8:0) inhibited the platelet shape change elicited by acyl-LPA (16:0 or 18:1) as well as alkyl-LPA (16:0). On testing other platelet-activating GPCR ligands, we found that DGPP(8:0) specifically inhibited shape change induced by LPA but not by other platelet stimuli, including PAF, the thromboxane receptor agonist U46619, ADP, serotonin, thrombin, and thrombin-receptor activating peptides (SFLRLRNP, YFLLRNPK) (Figure 1A and data not shown). Only the short-chain derivative DGPP(8:0) had an inhibitory effect, whereas the long-chain version DGPP(18:1) was inactive (Figures 1 and 2A).

Other analogs of LPA and PA were also tested for agonist and antagonist activity in human platelets (Table). Several compounds inhibited LPA-induced shape change, some with IC50 values lower than that of DGPP(8:0). However, all these analogs had agonist activity. This observation raised the possibility that these compounds might achieve their inhibition through desensitizing LPA receptors.

The effect of DGPP(8:0) on the inhibition of platelet shape change induced by 100 nmol/L of LPA was dose-dependent (Figure 2A). The IC50 of DGPP(8:0) was ~4-fold lower than that of PA(8:0) (Table). Using a constant concentration of DGPP(8:0) equivalent to its IC50 value (2.5 μmol/L), the LPA dose-response curve shifted to the right by a factor 3 (Figure 2B). Inhibition of LPA-induced shape changes by DGPP(8:0) required preincubation with platelets. The inhibitory effect increased with the time of preincubation, and a time period of 5 minutes was sufficient to achieve a maximal inhibition by 10 μmol/L DGPP(8:0) (data not shown). In washout experiments, we found that the inhibitory effect of DGPP(8:0) was completely reversible, indicating a lack of irreversible modification of platelet LPA receptors by DGPP(8:0).

DGPP(8:0) Inhibits Platelet Ca2+ Mobilization Induced by LPA

High concentrations of LPA (>1 μmol/L; EC50, 5 μmol/L) induce a small increase in cytosolic Ca2+ in platelets that is mainly caused by the stimulation of Ca2+ entry. We found that DGPP(8:0) (10 μmol/L for 2 minutes) specifically inhibited the increase of cytosolic Ca2+ elicited by LPA (10 μmol/L) by 80±5% (mean±SD, n=4) but not other platelet

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<th>% of Max SC</th>
<th>EC50 SD, μmol/L</th>
<th>IC50 SD, μmol/L</th>
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<tr>
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Shape change induced by 0.1 μmol/L LPA was set at 100%. Values are mean±SD from different experiments and platelet donors. SC indicates shape change.

Figure 1. DGPP(8:0) but not DGPP(18:1) inhibits specifically LPA-induced shape change. Suspensions of washed human platelets were incubated with solvent, 10 μmol/L of DGPP(8:0), or DGPP(18:1) for 2 minutes before exposure to LPA (100 nmol/L), PAF (100 nmol/L), U46619 (100 nmol/L), YFLLRNPK (300 μmol/L), and ADP (0.5 μmol/L). Shape change was recorded as decrease in light transmission. Tracings shown are representative for 5 experiments.
stimuli such as U46619 (100 nmol/L), PAF (20 nmol/L), or ADP (1 µmol/L) (data not shown).

**DGPP(8:0) Inhibits Platelet Shape Change Induced by Mox-LDL and the Lipid Core of Human Atherosclerotic Plaques**

Extending our previous observations that LPA is an important platelet-activating lipid in mox-LDL, we found that preincubation of platelets with DGPP(8:0) almost completely abolished shape change induced by mox-LDL (Figure 3A). Moreover, DGPP(8:0) completely inhibited platelet shape change induced by lipid extracts from the lipid-rich core of human carotid artery plaques isolated from patients with carotid stenosis (Figure 3A). These observations suggest that LPA receptor ligands are the primary platelet-activating lipids in mox-LDL and in the lipid core of human atherosclerotic plaques.

We discovered that the lipid-rich core isolated from human carotid plaques is capable of activating platelets. We observed that in 7 of 12 carotid atherosclerotic plaques obtained from different patients that this material consistently induced platelets to change their shape (Figure 3A, lower tracing; Figure 4C). This initial platelet response was blocked by preincubation with DGPP(8:0); subsequent addition of LPA was without effect, whereas the addition of ADP still induced shape change (Figure 3A, lower tracing). Quantitative measurements of the shape change responses induced by the active plaques showed that DGPP(8:0) almost completely inhibited this response. Another LPA receptor antagonist, NPSerPA, had the same effect (Figure 3B).

Morphological examination by confocal fluorescence microscopy of F-actin–stained platelets corroborated the functional observations: The lipid-rich core induced a reorganization of the actin cytoskeleton during platelet shape change similar to LPA. Pretreatment of platelets with DGPP(8:0) almost completely inhibited the morphological shape change induced by LPA or the lipid-rich core, as >90% of the cells were discoid (Figure 4).

These results indicate that LPA present in the vulnerable lipid-rich core region of atherosclerotic lesions rapidly trigger platelet shape change and that this early phase of platelet activation is efficiently blocked by the LPA₁ and LPA₂ receptor antagonist DGPP(8:0).

**Quantitative LC-MS Analysis and Comparison of Biological Activities of LPA Molecular Species in the Lipid Core of Human Atherosclerotic Plaques**

To identify and quantify the LPA molecular species present in atherosclerotic plaques, we performed LC-MS analysis, which has previously been shown to be accurate and sensitive for the determination of individual LPA -species in human plasma and serum.²⁷ The LC-MS analysis showed the presence of acyl-LPA (80%) and alkyl-LPA (20%) species in the lipid-rich core regions (Figure 5A). The major acyl-LPA species was LPA₁(18:0), contributing 58±9% of total acyl-LPA; the relative amounts of LPA₁(18:1), LPA₁(16:0), and LPA₁(20:4) were 18±7%, 10±3%, and 7±5%, respectively. In contrast, the predominant alkyl-LPA species LPA₂(18:1) (42±8%), LPA₂(18:0), and LPA₂(16:0) were less abundant (23±8 and 20±12, respectively) (mean±SD, n=6). Total

**Figure 2.** A, DGPP(8:0) inhibits LPA-induced shape change in a concentration-dependent manner. Platelets were incubated with solvent (control) or different concentrations of DGPP(8:0) or DGPP(18:1) for 10 minutes before exposure to LPA (100 nmol/L). Values are mean±SD, n=7. B, DGPP(8:0) inhibits LPA-induced shape change in a competitive manner. Platelets were incubated with DGPP(8:0) (2.5 µmol/L) or solvent for 2 minutes before exposure to different concentrations of LPA. Values are mean±SD, n=3.
LPA levels as estimated by bioassay on platelet shape change were similar to those determined by LC-MS. By comparing the biological effect of 3 molecular species of LPA found in the lipid-rich core, we found that alkyl-LPA (16:0) and acyl-LPA (20:4) were 18- and 6.5-fold more potent at inducing platelet shape change than acyl-LPA (16:0) (Figure 5B). Therefore these LPA molecules, although present in lesser amounts, might contribute significantly to the platelet-activating effect of the lipid rich core.

Synergistic Induction of Platelet Aggregation by LPA or Plaque Lipid-Rich Core With Subthreshold Concentrations of ADP or Epinephrine: Inhibition by DGPP(8:0)

Platelet aggregation that occurs subsequent to shape change is the main platelet response, which produces an occluding intravascular plug.28 We observed that coactivation of platelets with LPA or plaque lipid-rich core together with subthreshold concentrations of other platelet agonists synergistically induced platelet aggregation (Figure 6, A through C). When LPA was added to platelet suspensions containing fibrinogen together with low concentrations of ADP, that alone induced only shape change, or with epinephrine, that itself was devoid of agonist activity, rapid aggregation responses were observed. Platelet aggregation was almost completely inhibited when platelets were preincubated with DGPP(8:0), an inhibitor of platelet shape change induced by LPA, but not ADP (Figure 6, A and B). Similarly, exposure of platelets to the lipid-rich core primed platelets to aggregate: The subsequent addition of a low ADP dose that alone induced only shape change induced maximal platelet aggregation (Figure 6C). Platelet aggregation was blocked when platelets were preincubated with DGPP(8:0), which inhibited the platelet shape change induced by the lipid-rich core (see tracing in Figure 6C). The same observations were made when platelets were preincubated with another LPA receptor antagonist, NPSerPA. These results indicate that LPA species present in the lipid-rich core are capable of triggering platelet aggregation.

Discussion

Advanced atherosclerotic lesions become life-threatening through sudden plaque rupture with superimposed thrombosis. We found previously, by bioassay, that LPA-like compounds accumulate in human atherosclerotic plaques and that the lipid-rich core is the region of the carotid atherosclerotic lesion that had the highest content of LPA-like biological activity.4 In the current study, we applied mass spectrometry to the identification and quantification of the molecular species of LPA in this plaque region and found that in addition to different molecular species of acyl-LPA, substantial amounts of alkyl-LPA were present. Notably, 20% of total LPA was alkyl analogs. The biological potency of alkyl-LPA is much higher than the corresponding acyl species, as demonstrated for the alkyl-LPA (16:0) and acyl-LPA (16:0) (Figure 5B), which have the same hydrocarbon chain length but differ in the ether versus ester bond. Significant amounts of acyl-LPA (20:4) were also detected in the plaque lipid core. This particular LPA species had also a higher platelet-activating potency than acyl-LPA (16:0) and acyl-LPA (18:1), in agreement with recent observations.29 Therefore...
fore, the platelet-activating effect of the lipid-rich core of atherosclerotic plaques is determined not only by the total amount of LPA but is also greatly influenced by the alkyl-acyl ratio and the molecular species composition of LPA.

In the current study, we demonstrated for the first time that the lipid-rich core also triggered maximal platelet aggregation when platelets were costimulated with low concentrations of ADP, which alone only induced shape change. Platelet shape change and synergistic platelet aggregation triggered by the lipid core was completely inhibited by DGPP(8:0), a specific antagonist of the LPA-induced shape change, as shown in the current study. These results were mimicked by using a further LPA receptor antagonist, NPSerPA, indicating that these LPA receptor antagonists can abrogate the effects of not only LPA but also mox-LDL and core lipids. Our study not only provides mass spectrometric evidence for the presence of LPA species with high platelet-activating potency in soft atherosclerotic lesions but also the potential of these LPA molecules to trigger platelet activation within the context of the other lipid core components. Platelet activation by the lipid core material was observed in 7 of 12 soft plaques obtained from different patients. The reasons for the lack of platelet activation in some samples remains unclear, but several hypotheses can be considered, including the presence of endogenous platelet inhibitory substances and/or lower concentrations of LPA species with high platelet-activating potency in the inactive plaque samples.

In addition to LPA, other platelet agonists such as the stromal-derived factor-1 chemokine, cholesterol sulfate, and oxidized phosphatidylcholine molecules with PAF-like activity have previously been reported to be present in human atherosclerotic lesions. However, their contribution to the thrombogenic potential of atherosclerotic plaques is not known.

Our study indicates a key role for LPA and its receptors in inducing the initial phase of platelet activation by the plaque lipid core. Platelet aggregation, the main platelet response leading to an occluding intravascular plug, required the costimulation of platelets with LPA or lipid core and subthreshold concentrations of other platelet agonists. The "priming" of platelets by LPA or lipid core together with ADP or epinephrine was sufficient to produce full platelet aggregation. Although platelet aggregation is a complex process that involves various positive feedback loops, it becomes apparent that on coactivation of 2 platelet receptors, which couple to different G-proteins, their specific signal transduction pathways converge in inducing platelet aggregation. Costimulation of the G12/13 signaling pathway by LPA receptor activation and the G1 signaling pathway activated by ADP or epinephrine probably underlies the strong synergistic platelet aggregation response observed in the current study. Indeed, it has been demonstrated recently that the activation of G12/13 and G1-mediated signaling pathways elicits full activation of the fibrinogen receptor. We found that DGPP(8:0) and PA(8:0) selectively inhibit LPA-induced platelet shape change and Ca2+ mobilization, with DGPP(8:0) being more potent than PA(8:0). Both compounds were the only inhibitory phosphatidate analogs examined that were devoid of agonist activity (Table). The antagonist activity of DGPP(8:0) was critically dependent on the chain length of the fatty acyl chains in the sn-1 and sn-2 positions. The short-chain analog DGPP(8:0) was a potent inhibitor, whereas the long-chain analog DGPP(18:1) was inactive. LPA(8:0) lacked agonist or antagonist activity.
DGPP(8:0) and PA(8:0) have been shown to be selective antagonists of the LPA₁ and LPA₃ receptors but are ineffective on the LPA₁ receptor.²⁰ The complete inhibition of shape change and Ca²⁺ response by DGPP(8:0) indicates that LPA₁ and/or LPA₃ receptors are likely to mediate these LPA-elicited platelet responses. However, insect cells that overexpress LPA₁ or LPA₃ showed no preference for alkyl-LPA over the acyl-LPA analogs; hence this feature of the platelet response is inconsistent with that of LPA₁ and LPA₃ receptors.³³,³⁴ Tokumura and colleagues²⁹ noted that platelets from some donors did not respond to alkyl-LPA and suggested an as-yet unidentified receptor for this LPA species. We found that DGPP(8:0) inhibited the platelet responses elicited by acyl-LPA as well as alkyl-LPA.

It emerges that LPA is unique as a platelet stimulus that is important under pathophysiological conditions. Hence, we hypothesize that drugs selectively inhibiting platelet activation induced by LPA will be beneficial in preventing and treating cardiovascular disease without the major, bleeding-related side effects of currently used antiplatelet drugs. The platelet LPA receptor antagonist DGPP(8:0) described in the current study could serve as a lead structure for the further development of more potent compounds.

Acknowledgments

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References

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