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Regulation of vascular cell function by bioactive lysophospholipids

A. J. Morris[*], **M. Panchatcharam**[*], **H.Y. Cheng**[*], **L. Federico**[*], **Z. Fulkerson**[*], **S. Selim**[*], **D. Escalante-Acalde**[†], and **S.S. Smyth**[*,‡]

[*] The Gill Heart Institute, Division of Cardiovascular Medicine, 900 S. Limestone, Lexington, KY, USA, 40536-0200

[†] Division de Neurociencias, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Ciudad Universitaria

[‡] Veterans Affairs Medical Center, Lexington, KY, USA

Summary

Lysophosphatidic acid (LPA) and its sphingolipid homolog sphingosine 1-phosphate (S1P) and several other related molecules constitute a family of bioactive lipid phosphoric acids that function as receptor-active mediators with roles in cell growth, differentiation, inflammation, immunomodulation, apoptosis and development. LPA and S1P are present in physiologically relevant concentrations in the circulation. In isolated cell culture systems or animal models, these lipids exert a range of effects that suggest that S1P and LPA could play important roles in maintaining normal vascular homeostasis and in vascular injury responses. LPA and S1P act on a series of G protein–coupled receptors, and LPA may also be an endogenous regulator of PPARγ activity. In this review, we discuss potential roles for lysolipid signaling in the vasculature and mechanisms by which these bioactive lipids could contribute to cardiovascular disease.

Introduction

Lysophospholipids are derivatives of glycero- or sphingo-phospholipids lacking one radyl hydrocarbon chain (Figure 1). The major bioactive lysophospholipids with cell surface receptor-mediated effects are lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). LPA and S1P circulate in blood, where they are bound to plasma proteins, such as albumin, and found in lipoprotein particles. The biological activities of LPA and S1P are wide-ranging, and in some cases opposing, and include effects on cell growth, apoptosis, adhesion, migration, and invasion. Many of the effects of LPA and S1P are mediated by binding to G-protein-coupled receptors. Because of structural similarities, LPA and SIP receptors were initially classified together as members of the Endothelial Differentiation Gene (Edg) family. Subsequently, these receptors have been more rationally reclassified as LPA receptors and SIP receptors to denote their ligand specificities[1]. To date, five G-protein-coupled LPA receptors have been identified definitively, and evidence for at least three more has been presented [1, 2]. LPA1-3 belong to the original Edg class of receptors, whereas most of the more recently identified LPA receptors show greater sequence identity with purinergic receptors. Five documented SIP receptors exist (S1P1-5) [3]. Both LPA and SIP receptor family members activate downstream signals coupled to $G_{i/o}$, G_q, or $G_{12/13}$-mediated pathways[1,4]. LPA may also serve as an endogenous activator of PPARγ [5,6].

Disclosure of conflict of interest

The authors report not conflicts of interest.

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Generation of lysophospholipids in the circulation

The data suggest roles for multiple vascular cells in maintaining LPA and S1P homeostasis (Figure 1) with a single discrete mechanism of synthesis for each mediator in plasma, although alternative pathways likely occur in certain settings. In the case of LPA, the primary route of production in blood involves hydrolysis of lysophosphatidyl choline (LPC) by the secreted lysopholipase D (lysoPLD) autotaxin. Autotaxin/lysoPLD is a member of the ectonucleotide pyrophosphatase/phosphodiesterase (Enpp) family, designated Enpp2, with a unique ability to hydrolyze LPC. Genetic deletion of *Enpp2* in mice (*Enpp2*$^{-/-}$) is lethal embryonically due in part to vascular defects and failure of vessel maturation [7,8]. *Enpp2*$^{+/-}$ mice have approximately half of the normal circulating levels of autotaxin/lysoPLD and LPA. Transgenic overexpression of human *Enpp2* using the α1-anti-trypsin inhibitor promoter to drive expression in liver increases plasma autotaxin/lysoPLD activity and LPA levels [9]. These findings establish that autotaxin/lysoPLD regulates LPA levels in the blood. The substrate for autotaxin/lysoPLD activity is likely LPC produced during cholesterol esterification or from the action of A-type phospholipases (PLA) [10]. Activated platelets can produce LPA through the actions of PLAs, and LPA levels in serum prepared from platelet-rich plasma have been reported to be ~5-10–fold higher than in platelet-poor plasma. Circulating LPA levels can be lowered in rodents by making them thrombocytopenic [11]. Isolated platelets can generate LPA directly, but the quantities involved are too small to make a significant contribution to serum LPA levels. It may be that a primary role of platelets here is in localized LPA production by generation of a pool of LPC that can serve as a substrate for autotaxin/lysoPLD. Autotaxin/ lysoPLD has recently been shown to bind leukocyte integrins [12]. We have found that it can associate with activated platelets in a β3 integrin-dependent manner and accumulates in arterial thrombi [9]. This interaction could be very important for localized generation of LPA and/or targeting the enzyme to cells, membrane microvesicles or lipoproteins that are enriched in its substrates. It is notable that an early study identified a critical role for microparticles produced by activated platelets and Ca^{2+}-treated erythrocytes as a key intermediate in LPA production in the blood.

S1P is produced by 2 sphingosphine kinases (Sphk1 and Sphk2) [13]. The two murine sphingosine kinase genes appear redundant because inactivation of either gene produces animals with no discernable phenotype. However, concurrent inactivation of both genes results in early embryonic lethality. Recent genetic studies in mice revealed a role for erythrocytes in maintaining plasma S1P levels. Cyster, Couglin and colleagues [14] generated animals that survival to adulthood with no detectable SIP in circulation by conditional deletion of *Sphk1* in *Sphk2*$^{-/-}$ pups. They demonstrated that plasma S1P levels can be restored in the *Sphk1*- and *Sphk2*-deficient animals by transplant of normal bone marrow cells or by transfusion red blood cells but not platelets. This finding is essentially a genetic ratification of a contemporaneously published study conducted using human and mouse models demonstrating a key role for erythrocytes as a “buffer” system in the storage and release of S1P in the blood [15]. It is important to emphasize that erythrocytes are almost certainly not the sole source of circulating S1P, and a very recent report by Hla and colleagues identifies a role for vascular endothelial cells in the production of circulating S1P[16].

Metabolism of lysophospholipids in circulation

As with the synthetic pathway, degradation of LPA and S1P likely proceeds by several pathways including phospholipase-catalyzed deacylation or reacylation to form receptor-inactive free fatty acids or phosphatidic acid. LPA and S1P can also be inactivated by dephosphorylation by cell surface integral membrane enzymes termed lipid phosphate phosphatases (LPPs). LPPs are a family of integral membrane glycoproteins that localize to the cell surface with a topology that orients the active site towards the extracellular space

[17,18]. Overexpression studies in mammalian cells and genetic experiments in Drosophila suggest that LPP3, in particular, functions as regulator of both rapid and longer term LPA signaling responses. Whereas overexpression of *Lpp1* does not alter circulating LPA levels or cellular responses to LPA [19] and deficiency of *Lpp2* [20] in mice has no obvious phenotypic effect, an unexpected role for LPP3 in early vascular development was revealed by genetic inactivation in mice. *Lpp3* deletion in mice is embryonically lethal [21], which we have found is recapitulated by targeted deletion of LPP3 in endothelial cells. Targeted-deletion of LPP3 in smooth muscle cells (SMC) reduces LPA degradation and increases migratory potential. A more selective S1P phosphatase enzyme also exists although this enzyme appears to be intracellular and likely plays a role in intracellular sphingolipid metabolism. S1P can also be degraded by an S1P lyase which is a pyridoxal phosphate-dependent enzyme that cleaves S1P at the C2-C3 bond to yield ethanolamine phosphate and hexedecenal [22].

Regulation of endothelial barrier function by lysophospholipids

The first defined lysolipid receptor S1P1/Edg1 was originally identified as a gene upregulated in endothelial cells in an *in vitro* model of angiogenesis. The identification of S1P as high-affinity ligand for this receptor provoked great interest in bioactive lysolipids as regulators of endothelial cell function and vasculature dynamics. Genetic inactivation of S1P1 in mice results in embryonic lethality and impaired vessel formation due to a lack of homing of SMC to the developing vessels [23]. In cultured cells, physiologic concentrations of S1P (0.5 – 1 μM) promote endothelial barrier functions via S1P1-mediated activation of the Rho GTPase Rac and cytoskeletal reorganization and stabilization of junctions [24,25]. S1P can restore barrier function after disruption by edemagenic agents such as thrombin. Additionally, many barrier-stabilizing agents, including activated protein C, transactivate S1P1 signaling. The potent protective effects of S1P on endothelial barrier function have led to the proposal that the lipid could serve as a therapy to reduce endothelial permeability in inflammatory settings such as sepsis and acute lung injury. In that regard, S1P is cardioprotective in animal models of ischemia/reperfusion injury [26]. Exposure of endothelial cells to higher concentrations of S1P (~ 5 mM) triggers S1P3-mediated Rho activation that disrupts barrier function. Recently, a protective effect for Sphk1 in maintaining endothelial barrier function in the setting of LPS- or thrombin-induced pulmonary edema has been demonstrated [27].

The effects of LPA on endothelial cell function have not been studied as extensively as SIP. The preponderance of the data would suggest that LPA elicits a loss of vascular integrity and decreases transendothelial resistance by preventing tight junction formation [28], although some investigators have observed that LPA stabilizes endothelial barrier function [29]. In a lung injury model, the LPA1 receptor mediates vascular leak [30].

Immunomodulatory properties of lysophospholipids

S1P promotes a range of physiologically relevant immunomodulatory effects [31]. The pharmaceutical compound FTY720, a pro-drug that is phosphorylated *in vivo* to produce an SIP receptor agonist and desensitizes S1P1, S1P2, and S1P5 receptors, prevents lymphocyte egress from thymus and lymphoid organs. Extensive data analyzing effects of FTY720 and results from mice lacking specific S1P receptors have established a role for S1P signaling and the S1P1 receptor, in particular, in regulation of T and B cell trafficking between the lymphatic system and the peripheral circulation. These studies support a model in which a gradient of S1P – high levels in blood and lymph and low levels in lymphoid organs – promotes the outward migration of lymphocytes. Recently Keul et al. demonstrated that FTY720 attenuates atherosclerosis formation in *ApoE*$^{-/-}$ mice and reduces macrophage accumulation in plaque [32]. It is noteworthy that S1P also prevents macrophage activation [33] and monocyte-endothelial interactions [34].

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Less is known about the immunomodulatory/inflammatory effects of LPA [35,36]. LPA receptors are found on lymphocytes, dendritic cells, and in lymphoid organs. Recent work by Rosen and colleagues demonstrated that autotaxin/lysoPLD binds to lymphocytes in an integrin-dependent manner to generate LPA at the cell surface which promotes the entry of lymphocytes into lymphoid organs [12]. LPA also stimulates chemotaxis of human dendritic cells and, in the case of immature murine dendritic cells, the effects are mediated by LPA3. LPA activates innate immune cells, including neutrophils, eosinophils, and mononuclear phagocytes.

Regulation of SMC function and the development of intimal hyperplasia by lysophospholipids

LPA has been proposed to be a key factor in serum that promotes phenotypic modulation of SMCs [37]. When added to cultured SMCs, LPA promotes dedifferentiation, proliferation, and migration Local infusion of LPA in the rodent carotid artery induces vascular remodeling by stimulating neointimal formation that may mediated by PPARγ [38]. LPA1 and LPA2 appear to be to be critical receptors for regulating SMC migration, however, the receptors that are coupled to other aspects of phenotypic modulation of SMC are not known. Additionally, LPA has vasoregulatory properties. For example, intravenous injection of LPA elevates arterial blood pressure in rats and mice and local application causes cerebral vasoconstriction in pigs.

Following arterial injury, an early increase in S1P1 and S1P3 levels occur in the vessel accompanied by a transient decrease in S1P2 levels, which is followed by a late increase in S1P2 levels [39]. Rat pup intimal SMC, which have a higher proliferative capacity, also express greater levels of S1P1 than do adult medial cells [40]. Mouse strains with higher S1P1 expression develop more intimal hyperplasia following arterial injury [41], and pharmacologically targeting of S1P1 and S1P3 reduces the formation of neotintima [39]. S1P1 and S1P3 may mediate their effects in part by promoting dedifferentiation of SMC. In contrast, S1P2 appears to protect from the development of intimal hyperplasia, in that *S1p2*-/- mice develop more robust neointima after arterial injury and an S1P2 antagonist promotes a more differentiated phenotype in SMC [42]. S1P may also regulate vascular tone, although in some arterial beds, it has vasoconstrictor properties and in other beds promotes vasodilation.

Atherothrombosis

While the studies related above, including those describing the effects of FTY720, suggest that S1P may be atheroprotective, the available data would indicate that LPA signaling could promote the development of atherosclerosis and its complications. Local concentrations of LPA may be increased along inflamed vessels and at sites of platelet adhesion and thrombus formation where autotaxin accumulates. Seiss and Tigyi found that LPA is abundant in the lipid-rich core of human atherosclerotic plaque, where is may be derived from mildly oxidized LDL [43]. We have more recently found that when normalized to total phospholipid phosphorous LPA and compared to healthy tissue, polyunsaturated LPA species are significantly enriched in experimentally induced murine atheromas. Thus, LPA is present or can be formed in the settings where it could influence development and complications of atherosclerosis. LPA triggers an inflammatory response in endothelial cells involving LPA1 and LPA3- mediated expression of leukocyte chemoattractants and adhesion receptors[44, 45]. These responses promote monocyte binding to endothelial cells. Moreover, LPA is a weak activator of platelets from most human donors [46], although it lacks stimulatory effects on rodent platelets. Thus, in addition to proinflammatory changes that may promote atherosclerosis, LPA exposure could contribute to the major complication of atherosclerosis, namely acute arterial thrombosis. In this regard, it is interesting that mouse platelets are not

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activated by physiologically relevant concentrations of LPA and that mice do not display spontaneous thrombosis even in the presence of extensive atherosclerosis.

Summary

The ongoing work discussed above clearly implicates both LPA and S1P in a wide range of cardiovascular functions. However, functional redundancy between different members of the LPA and S1P selective receptor classes coupled with compensatory changes in their expression continues to make it challenging to use mice with targeted inactivation of these receptor genes to provide definitive insights into the roles of these lipids in cardiovascular physiology and disease. Interestingly, the processes involved in LPA and S1P production and metabolism appear to involve fewer non-redundant genes. For example, LPA production in the blood is critically dependent on a single gene product, autotaxin/lysoPLD and manipulation of this enzyme in mouse models has significant effects on circulating LPA levels. Efforts aimed at targeting LPA and S1P synthesis and inactivation may prove to be more effective strategies for both experimental investigations into their roles in cardiovascular function and disease in animal models and eventually for pharmacological intervention in humans.

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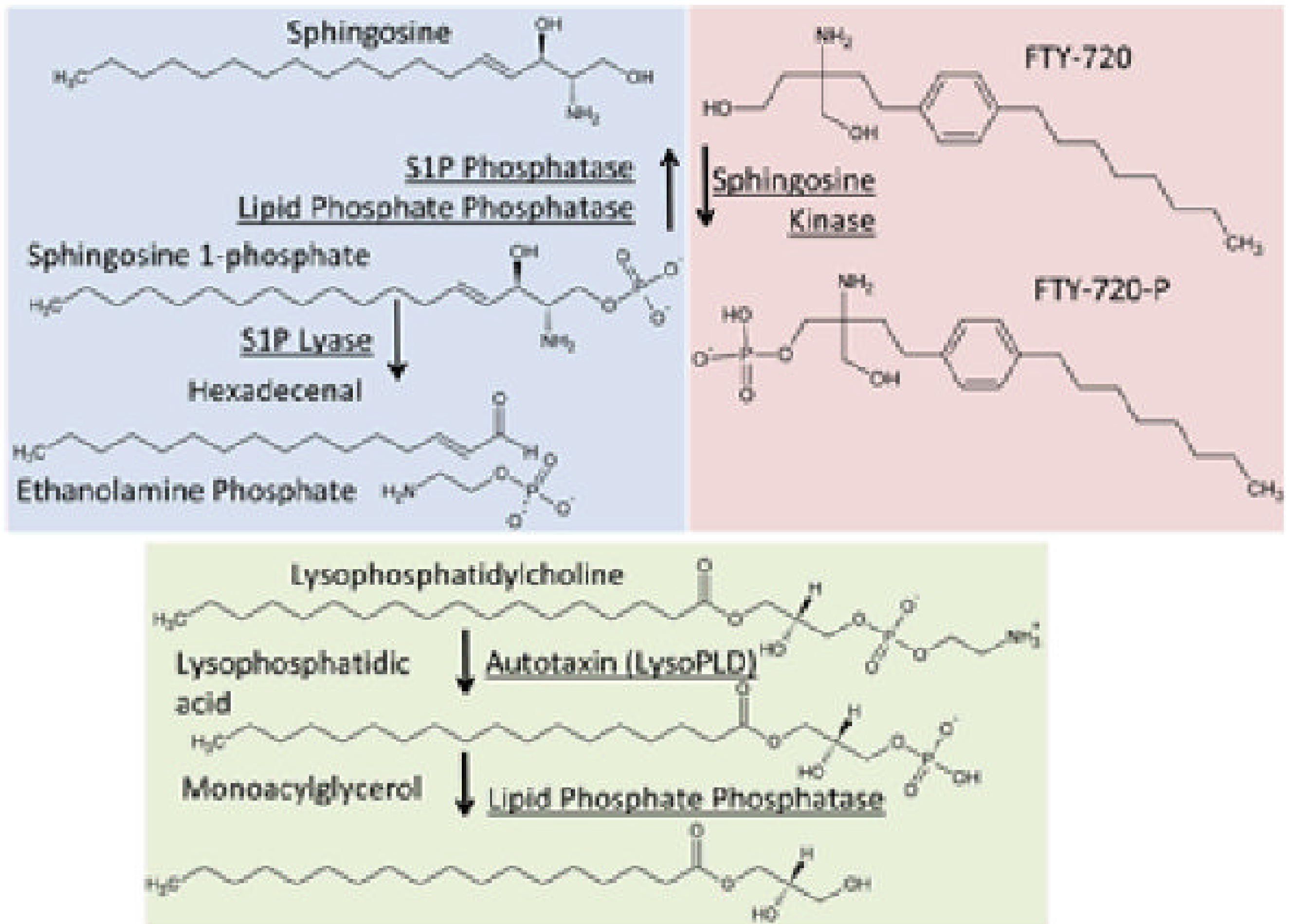

Figure 1. Synthesis and inactivation of S1P and LPA

S1P is synthesized intracellularly by sphingosine kinase –catalyzed phosphorylation of sphingosine. Note that dihydrophingosine is also a substrate for this enzyme which forms dihydro S1P. These dihydro derivatives lack the double bond between the C4 and C5 carbon atoms. S1P can be converted to sphingosine by dephosphorylation catalyzed by a selective S1P phosphatase or the broader specificity lipid phosphate phosphatases. S1P can also be degraded by S1P lyase to form hexadecenal and ethanolamine phosphate. The predominant pathway for the production of extracellular LPA is from lysophospholipids, most likely lysophosphatidylcholine by the lysophospholipase D activity of autotaxin. LPA is inactivated by dephosphorylation catalyzed by the broad specificity lipid phosphate phosphatases to form monoacylglycerol. LPA can also be made by phospholipase A2 catalysed hydrolysis of phosphatidic acid. Intracellularly, LPA can be made *de novo* by acylation of glycerol 3-phosphate for synthesis of di- and tri-glycerides and phospholipids.

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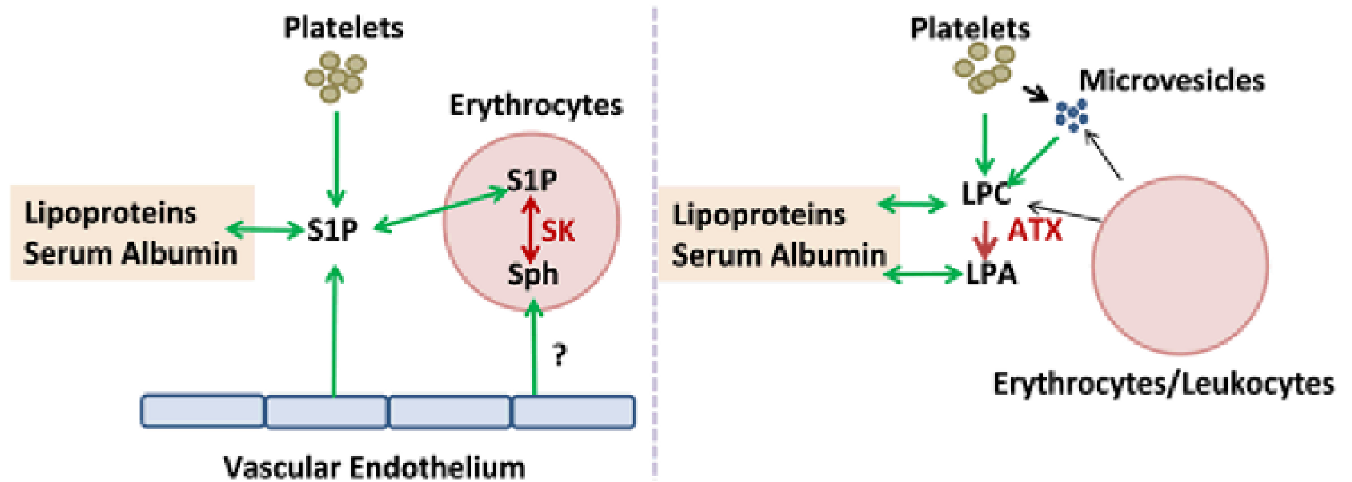

Figure 2. S1P and LPA homeostasis in the blood

S1P can be produced by platelets, vascular endothelium and erythrocytes. Adoptive transfer and transfusion experiments establish a critical role for erythrocytes in this process in mice. Erythrocytes sphingosine kinase and *de novo* synthesis of S1P may involve uptake of sphingosine (sph) from an undefined source, possibly vascular endothelium. S1P is carried in the blood bound to lipoproteins and serum albumin. LPA is formed by lysophospholipase D activity of autotaxin (ATX) which hydrolyzes circulating lyosphospholipids, predominantly LPC which may be formed as a by-product of cholesterol esterification or potentially generated by phospholipases acting on lipids in platelets, erythrocytes or membrane microparticles released from these cells. Like S1P, LPA and LPC are bound to serum albumin and lipoproteins