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Lysophospholipids and their G protein-coupled receptors in atherosclerosis

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Abstract

Lysophospholipids (LPLs) are bioactive lipid-derived signaling molecules generated by the enzymatic and chemical processes of regiospecific phospholipases on substrates such as membrane phospholipids (PLs) and sphingolipids (SLs). They play a major role as extracellular mediators by activating G-protein coupled receptors (GPCRs) and stimulating diverse cellular responses from their signaling pathways. LPLs are involved in various pathologies of the vasculature system including coronary heart disease and hypertension. Many studies suggest the importance of LPLs in their association with the development of atherosclerosis, a chronic and severe vascular disease. This paper focuses on the pathophysiological effects of different lysophospholipids on atherosclerosis, which may promote the pathogenesis of myocardial infarction and strokes. Their atherogenic biological activities take place in vascular endothelial cells, vascular smooth muscle cells, fibroblasts, monocytes and macrophages, dendritic cells, T-lymphocytes, platelets, etc.

Keywords

Lysophospholipids; G protein-coupled receptors; Vascular inflammation; Atherosclerosis; Review

2. INTRODUCTION

LPLs, as metabolic intermediates in biosynthesis of membrane phospholipids, play a major role as extracellular mediators by activating GPCRs and stimulating diverse cellular responses from their signaling pathways. They include well-characterized lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), which play important roles in not only

physiological but also pathophysiological processes including smooth muscle contraction, proliferation, pain, inflammation, atherosclerosis, myocardial injury, cancer cell migration and invasion, etc. Many studies suggest the importance of LPLs in their association with the development of atherosclerosis, a chronic and severe vascular disease. More and more G protein-coupled LPLs receptors (LPL-GPCRs) have been identified, and it has been studied that more species of LPLs, such as lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS), lysophosphatidylethanolamine (LPE) and sphingosylphosphorylcholine (SPC) are involved in atherosclerosis. However there are few review papers summarizing LPL-GPCRs signaling pathways or focusing on the pathophysiological effects of different species of LPLs on atherosclerosis. In this paper, we summarized the LPL-GPCRs signaling pathways, biosynthesis of LPLs, bio-functions of LPLs and discussed the roles of LPLs in the development and progression of vascular inflammation and atherosclerosis.

3. LYSOPHOSPHOLIPIDS

3.1. LPLs cell signaling

LPLs are bioactive lipid derivatives, which are generated via the actions of phospholipase on major membrane PLs and SLs. Accordingly, LPLs are divided into two categories: glyceryl-based LPLs (glyceryl-LPLs) and sphingosyl-based LPLs (sphingosyl-LPLs), which have either a glycerol or a sphingosine backbone, respectively (1–3). However, they are all characterized by having a single tail of carbon chain (fatty acid residue) and a polar head group. All of these structural characteristics make them more hydrophilic and versatile than their precursors (4). The hydrophobic tail and the hydrophilic head group determine the specific chemical structures of LPLs and consequently affect their unique biological activities (5, 6) (Figure 1).

Originally, the LPLs were considered as metabolic intermediates in the biosynthesis of various biological membrane phospholipids. However, subsequent studies demonstrated that the LPLs exhibit wide-ranging biological properties. They include well-characterized LPA and S1P, which play important roles in not only physiological but also pathophysiological processes including smooth muscle contraction, proliferation, pain, inflammation, atherosclerosis, myocardial injury, cancer cell migration and invasion, etc. (7–11). The LPLs act as signaling mediators by binding to seven-transmembrane domain GPCRs and either activating or inhibiting downstream secondary messengers including Rho-associated kinase (Rock), diacylglycerol (DAG), inositol 1,4,5-trisphosphate (IP3), mitogen-activated protein kinase (MAPK), Ca^{2+} , adenylate cyclase (AC), phosphoinositide-3-kinase (PI3K), etc. (12–14) (Figure 2, Suppl. Table 1).

3.2. G protein-coupled LPLs receptors (LPL-GPCRs)

Most of the biological effects of LPLs are mediated by GPCRs. In 1996, the first LPL-GPCR was identified and shown to be LPA receptor (15). Until now, more than 26 GPCRs have been identified as LPLs receptors (12, 16–20) (Table 1). Because several of the LPL-GPCRs were independently identified in unrelated studies, there are historically several different names for some LPL-GPCRs. In particular, a group of proteins that are originally identified as GPCRs and coded by endothelial differentiation genes (EDGs) are later found

to be the same as several of the LPLs receptors. For example, LPA₁ is also known as EDG-2 (21). With the progress made for LPL-GPCRs, a broader range of physiological and pathophysiological effects of LPLs has been identified. LPL-GPCRs can regulate cell proliferation and survival, migration and chemotaxis, cytoskeletal architecture, cell-cell contacts and adhesion, Ca²⁺ homeostasis and Ca²⁺-dependent functions, etc. (4, 5, 13, 14, 22). Intelligibly, LPLs have been implicated in atherosclerosis (1, 10, 19, 23).

4. BIOSYNTHESIS OF LYSOPHOSPHOLIPIDS

4.1. Phospholipids biosynthesis

Natural PLs and SLs are the natural precursors of LPLs, and phospholipases play crucial roles in the biosynthesis and metabolism of PLs. To better understand the biosynthesis of LPLs, we would first discuss the biosynthesis of PLs and SLs.

4.1.1. PLs biosynthesis—Phospholipids are a class of lipids that consist of two fatty acyl molecules esterified at the sn-1 and sn-2 positions of glycerol, and contain a head group linked by a phosphate residue at the sn-3 position (Figure 3). Phospholipids are the main constituent of biological membranes. The size, shape, charge, and chemical composition of different phospholipid classes play a role in the formation and maintenance of the plasma membrane bilayer of cells, as well as membranes surrounding subcellular organelles and vesicles.

Phosphatidic acid (PA) forms the backbone where the synthesis of other phospholipid species and triacylglycerol is based. PA synthesis begins with the addition of a fatty acyl-CoA, usually saturated, to glycerol 3-phosphate at the sn-1 position to produce LPA. This reaction is catalyzed by glycerol 3-phosphate acyltransferase and is a rate-limiting step for PA synthesis. There are two forms of this enzyme; one is found in the outer mitochondrial membrane, while the other is found in the endoplasmic reticulum. A second fatty acyl-CoA, often unsaturated, is added to LPA at the sn-2 position by acylglycerol-3-acyltransferase to form phosphatidic acid. This occurs primarily in the endoplasmic reticulum.

PA can be used in the synthesis of several phospholipids by two different mechanisms. In brief, the hydrolysis of PA by the enzyme phosphatidate phosphatase results in sn-1,2-diacylglycerols (DAG), which are the precursors for the biosynthesis of triacylglycerols (TAG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) via the so-called Kennedy pathway. Via the reaction with cytidine triphosphate, PA serves as the precursor of cytidine diphosphate diacylglycerol (CDP-DAG), which is the key intermediate in the synthesis of phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS). Depending on the organism and other factors, phosphatidylserine can serve as a precursor for phosphatidylethanolamine. Similarly, the latter can give rise to phosphatidylcholine by the pathway of mono- and dimethyl-phosphatidylethanolamine intermediates (Figure 4).

4.1.2. SLs biosynthesis—The SLs, like the phospholipids, are composed of a polar head group and two nonpolar tails. The core of sphingolipids is the long-chain amino alcohol, sphingosine. The sphingolipids include the sphingomyelins and glycosphingolipids (Figure

3). Sphingomyelins are the only sphingolipids class and also phospholipids. Glycosphingolipids, including cerebrosides, sulfatides, globosides and gangliosides, are not phospholipids.

Ceramides, which consist of a long-chain or sphingoid base linked to a fatty acid via an amide bond, can be produced by *de novo* synthesis through the concerted action of serine palmitoyltransferase and dihydroceramide synthase. It can also be generated through the metabolism of more complex sphingolipids. Ceramide can be metabolized to ceramide-1-phosphate by ceramide kinase or to glucosylceramide by glucosylceramide synthase (GCS). The reverse reaction is catalyzed by ceramide-1-phosphate phosphatase, or by lipid phosphate phosphatases. Alternatively, ceramide can be degraded by ceramidases to form sphingosine, which can, in turn, be phosphorylated to S1P by sphingosine kinase. The reverse reaction is catalyzed by S1P phosphatases, or by lipid phosphate phosphatases. Sphingomyelin N-deacylase generates sphingosylphosphorylcholine (Figure 4).

4.2. Glyceryl-LPLs biosynthesis

As we discussed above, LPA can be produced by the enzyme-mediated *de novo* synthesis from fromglycerol-3-phosphate and fatty acyl-CoA. Most of the glyceryl-LPLs are produced through phospholipases-mediated hydrolysis of one acyl group of PLs.

Phospholipases form a diverse class of enzymes optimized to hydrolyze phospholipid substrates at specific ester bonds. Phospholipases vary considerably in structure and function, and as such they are assembled as a group of lipolytic enzymes solely involved in PL metabolism. Two general sets of phospholipases exist, the acyl hydrolases and the phosphodiesterases. The enzymes within each set are classified according to the cleavage of the ester bond for which they are specific (Figure 5). Phospholipase A1 (PLA1), phospholipase A2 (PLA2), phospholipase B (PLB), and lysophospholipase A1/2 (LysoPLA1/2) constitute the acyl hydrolases, whereas the phosphodiesterases are represented by phospholipase C (PLC) and phospholipase D (PLD). The PLA family of lipases consists of the PLA1 and PLA2 subfamilies. PLA1 enzymes catalyze the hydrolysis of fatty acids from the sn-1 position of glycerophospholipids in generating 2-acyl-lysophospholipids and free fatty acids. PLA2 enzymes catalyze the hydrolysis of the sn-2 position of glycerophospholipids releasing free fatty acids and 1-acyl-lysophospholipids.

The mammalian genome contains more than 30 genes encoding PLA2 and PLA2-related enzymes. All of these genes are subdivided into several classes that includes low molecular weight secreted PLA2s (sPLA2s), Ca²⁺-dependent cytosolic PLA2s (cPLA2s), Ca²⁺-independent PLA2s (iPLA2s), platelet-activating factor acetylhydrolases (PAF-AHs), lysosomal PLA2s, and a recently identified adipose-specific PLA2 (AdPLA). The intracellular cPLA2 and iPLA2 families and the extracellular sPLA2 family are recognized as the most significant PLA2 enzyme families (Suppl. Table 2).

4.3. Sphingosyl-LPLs biosynthesis

4.3.1. S1P biosynthesis—The synthesis of S1P occurs exclusively from sphingosine via the action of sphingosine kinases as we described above.

4.3.2. SPC, SPE, and SPI biosynthesis—Sphingosylphosphorylcholine (SPC), sphingosylphosphoethanolamine (SPE), and sphingosylphosphoinositol (SPI) can be produced by *N*-deacylase from Sphingomyelin, ceramide phosphorylethanolamine, and ceramide phosphorylinositol, respectively. A few studies in this field have been reported.

5. BIO-FUNCTIONS OF LPLS

Lysophospholipids, generated and regulated by the specific enzymes, play a significant role in the physiological and pathophysiological processes. LPLs activate a wide range of GPCRs for the proper functions and the development of cardiovascular, immune, respiratory, reproductive and nervous systems. Specific LPL receptors are involved in metabolism, inflammation, arteriosclerosis, atherosclerosis, and cancer. This is due to the regulation of LPL-GPCRs on cell proliferation and survival, cell migration and adhesion, angiogenesis, membrane process retraction, platelet aggression, Ca^{2+} homeostasis, and cytoskeleton architecture (4, 24, 25).

LPLs, such as LPA and S1P, have been well studied; and their GPCR signaling pathways lead to a cascade of various responses in many cells. LPA and S1P are intercellular biologically active lipid mediators expressed in many tissues and cell types (25). LPA signaling plays a key role in tumor progression, vascular development, endothelial integrity, and lymphocyte homing. LPA also has apoptotic action in macrophages and Schwann cells, which are glial cells that encase the axons (26). S1P inhibits the cell migration IL-8-stimulated neutrophils and invasion of melanoma cells (24). Overall, LPLs control cellular activities and are expressed throughout the body's biological systems in diverse and extensive ways.

6. ROLES OF LYSOPHOSPHOLIPIDS IN ATHEROSCLEROSIS

Atherosclerosis (AS) is a chronic and severe arterial disease, and involves the hardening of the arteries and the formation of plaques. Atherosclerosis is a key pathogenic cause underlying myocardial infarction (heart attacks) and strokes. The plaques narrow the arterial lumen, which reduces the oxygen supply of affected artery controlled tissues. This can result into tissue ischemia of the brain or myocardium. The atherogenic vascular disease is characterized by cells involved in inflammatory and regenerative/remodeling processes which reside in medium- to large-sized arteries such as aortic, coronary and cerebral arteries. The accumulation of activated endothelial cells (ECs), proliferative/synthetic vascular smooth muscle cells (VSMCs), fibroblasts, inflammatory monocytes and macrophages, monocyte-derived dendritic cells (DCs), and platelets in the arteries contribute to progression of atherosclerosis (10, 27, 28).

6.1. LPA and AS

LPA, lysophosphatidic acid, (1-acyl 2-hydroxyl glycerol 3-phosphate), which is a basic phospholipid, acts as a lipid mediator in the pathogenesis of atherosclerosis. LPA is an autocoid, which forms and acts near its production site on demand. LPA is formed during the oxidation of low density lipoprotein (LDL) and accumulates in elevated concentrations in atherosclerotic lesions. After the vicinal formation of the simple phospholipid, LPA

activates cells in the local area in response to risk signals such as a vascular injury or inflammation. This is especially important because high levels of oxidized LDL (oxLDL) are a key risk factor for cardiovascular disease, and play a significant role in the atherogenesis. The lysophospholipid partakes in most of the atherogenic processes as it was discovered to affect blood vessel function and alter cerebrovascular activity. LPA mediates biological activities on major cellular contributors in atherosclerosis including ECs, VSMCs, fibroblasts, monocytes, macrophages, dendritic cells, T-lymphocytes and platelets. The binding of cell surface GPCRs and activation of nuclear receptor PPAR γ signaling result from the action of LPA (10, 27, 29).

6.1.1. The effects of LPA on vascular ECs—Vascular ECs are the cells that form the endothelium, the lining of entire circulatory system. One of the initial steps of atherosclerosis is the activation of ECs, which initiates the formation of atherosclerotic lesions in the intima of susceptible arteries (10, 27). In endothelial cells, LPA induces cell migration, adhesion molecule expression, chemokine secretion, angiogenesis, and affects vessel permeability. The LPA-induced expression of adhesion molecules on the surface of ECs such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) increases the movement and proliferation of monocytes, macrophages and lymphocyte cells onto intima layer of vascular wall under the endothelium. This proinflammatory cellular accumulation initiates the atherosclerotic process by beginning the buildup of plaques. LPA also stimulates ECs to increase secretion of proinflammatory cytokines and chemokines such as interleukin-1 β (IL-1 β), IL-8 and monocyte chemo-attractant protein (MCP)-1. This, in turn, will enhance the generation of cytokines/chemokines gradients, promote monocyte cell recruitment to the arteries and facilitate the progression of atherosclerosis (30). Although not thoroughly studied, the induction of pentraxin-3 secretion by LPA enhances monocyte migration as well, suggesting a contribution to atherosclerotic development (31).

In addition, LPA stimulates angiogenesis. Angiogenesis is the formation of new blood vessels from preexisting vessels facilitated by ECs proliferation and migration. Angiogenesis can increase the advancement of atherosclerotic plaques due to its contribution to newly formed intraplaque microvessels, growth and instability. Moreover, LPA has an effect on increasing EC permeability due to its disruption of the endothelial barrier, although the results in this aspect remain controversial. Conflicting studies show several variable factors including endothelial heterogeneity, which can both increase and decrease endothelial barrier function leading to the onset and progression of atherosclerosis (32, 33).

6.1.2. The effects of LPA on VSMCs—LPA can cause phenotype changes in VSMCs located near the intima, by transforming VSMCs to a synthetic phenotype with less contractility and more proliferation and migration. This is significant in the pathogenesis of atherosclerosis because these intimal VSMCs are the prevailing cell type in atherosclerotic lesions and contribute significantly to the thickening of the artery (neointima). LPA induces the migration and proliferation of VSMCs and is an effective inducer of VSMCs de-differentiation (34). LPA promotes VSMCs release of inflammatory cytokines and chemokines such as IL-6 and MCP-1. These proinflammatory mediators can enter

circulation, which will, in effect, worsen atherosclerotic lesions. For example, LPA-induced MCP-1 in intimal VSMCs can direct trans-endothelial migrations of adherent monocytes and T-lymphocytes and passage into the arterial intima, which will promote atherogenesis (35).

6.1.3. The effects of LPA on fibroblasts—Although not many studies have been conducted to analyze LPA's effects on fibroblasts, it was found that LPA induces fibroblast cell migration and proliferation (36). An excessive amount of fibroblast cell production and migration can contribute to atherogenesis (37).

6.1.4. The effects of LPA on monocytes and macrophages—Monocytes and macrophages are important components in the development of atherosclerosis. One of the first steps for the early development of atherosclerosis is the migration of monocytes to the sub-endothelial space of the vascular wall (27). LPA increases monocyte migration, and favors monocyte retention in plaques and foam cell formation in the sub-endothelium of arterial wall (29). LPA enhances monocyte migration by inducing the secretion of endothelial chemokine (C-X-C motif) ligand 1 (CXCL1) and pentraxin-3 (31). LPA also induces endothelial cells to secrete IL-8 and MCP-1, which suggests that LPA indirectly activates cytokine receptors of monocytes (30). LPA may worsen lesions by modulating monocyte proinflammatory activation. LPA produces reactive oxidative intermediates (ROI) and induces monocyte cells to release proinflammatory arachidonic acid and prostaglandin E2, which are involved in the early activation of monocyte cells and subclinical atherosclerosis, respectively.

Furthermore, LPA plays a significant role in activating macrophages, thereby promoting the early stages of atherosclerosis. Plaque formation can arise prematurely in atherosclerosis because LPA can activate PI3K, which protects macrophages from undergoing apoptosis (10). LPA induces macrophages to be inclined to take up oxLDL, forming foam cells and thus leading to lesion formation (38). Overall, the role of LPA on macrophages and monocytes can promote the premature progress of atherosclerosis.

6.1.4. The effects of LPA on DCs—DCs are a type of monocyte-derived cells. LPA enhances DC maturation and assists in recruiting these immature cells into the subendothelium, associating them with atherosclerosis (39).

6.1.5. The effects of LPA on T lymphocytes—LPA promotes lymphocyte proliferation, migration and IL-2 production. It also suppresses T-lymphocytes from undergoing apoptosis and induces the secretion of proatherogenic cytokines (40). The role that LPA has on these cells may be related to the early stages of the atherogenesis.

6.1.6. The effects of LPA on platelets—The effects of LPA on platelets led to the early discoveries that LPA affects isolated platelets. LPA and platelets display positive interaction, in which activated platelets release the lysophospholipid and then the released LPA in return promotes platelet activation, shape change, and aggregation. LPA signaling has a possible role in facilitating the development of atherosclerosis due to its many strong effects on the immune/blood cell reactions (41).

6.2. LPC and AS

Lysophosphatidylcholine (LPC) is an important cell signaling molecule that is generated by phospholipase A2 on the substrate phosphatidylcholine. LPC is a ligand for specific GPCRs such as G2A and GPR4 in addition to its activation of several secondary messengers. The G2A receptor promotes the migration of immune cells and plays a role in enhancing apoptosis of lymphocytes and macrophage cells (42). GPR4 enhances the expression of adhesion molecules in endothelial cells involved in the migration of inflammatory cells, and impairs endothelial barrier functions, which contribute to atherogenesis (43).

LPC has a high concentration in human blood, and is the most abundant LPL. Many of the circulating LPC molecules are bound to albumin and some are bound to lipoprotein molecules. Depending on the cell types of arteries and oxidative and inflammatory states, it was found that LPC has both pro- and anti-atherogenic roles (19). LPC has extensive research on its pro-atherogenic roles; it activates signal-transduction cascades involved in the initiation and progression of atherosclerosis (44).

LPC's pro-atherogenic effects are as follows: *first*, LPC up-regulates MCP-1 and EC adhesive molecules such as ICAM-1 and VCAM-1; *second*, LPC mediates vascular cell remodeling and induces the secretion of proinflammatory cytokines; *third*, LPC enhances T-cell recruitment and causes apoptosis; *fourth*, LPC enhances monocyte cell proliferation and migration; and *fifth*, LPC drives pro-inflammatory mechanisms as a component of oxLDL (19).

6.2.1. The effects of LPC on ECs—LPC stimulates the activation of endothelial cells, which attracts immune cells to adhere to the vascular endothelial wall. LPC can initiate the gene transcription of endothelial cells and induce the expression of endothelial nitric oxide synthase (eNOS), MCP-1, VCAM-1, ICAM-1, P-selectin, growth factors and many more atherosclerotic contributors. Endothelial cells are stimulated to produce reaction oxygen species (ROS) and increase eNOS expression in atherosclerosis. Through the mechanisms involved in the protein kinase C (PKC)-mediated pathway, LPC can modulate the DNA-binding activities of proinflammatory transcription factors such as NF- κ B and AP-1, which will upregulate the expression of endothelial genes involved in atherosclerosis. For example, LPC can stimulate PKC, leading to the increased expression of ICAM-1 and attracting leukocyte adhesion on ECs (44).

A critical step in atherogenesis is the adhesion of blood monocytes to the endothelium, subsequently this type of leukocytes will be induced to perform trans-endothelial migration to the subendothelial space of the arteries. Due to the expression of molecules such as ICAM-1 and VCAM-1, the migration of monocytes, macrophages, and lymphocytes can be realized. These migrated cells generate different proinflammatory cytokines, which will in return further activate local ECs to produce adhesion molecules. This indicates that LPC may have a stimulating role in recruiting immune cells into the arterial intima during atherogenesis (45).

6.2.2. The effects of LPC on VSMCs—Vascular smooth muscle cells can change phenotype from a contractile to a secretory/synthetic phenotype. This is an important

characteristic of atherogenesis. LPC mediates vascular cell remodeling due to its role in inflammation and cell proliferation. LPC contribution to inflammation, vascular smooth muscle cell proliferation, and stimulation of remodeling pathways are caused by the inductive effects of LPC on the secretion of proinflammatory cytokines IL-6, IL-8 and granulocyte-macrophage colony stimulating factor (46).

6.2.3. The effects of LPC on T-Cells—T-cells, also known as T-lymphocytes, are found in the beginning and end stages of atherosclerotic lesions. LPC enhances T-cell recruitment and apoptosis in atherosclerotic lesions, contributing to the progression of atherosclerosis (47).

6.2.4. The effects of LPC on monocytes and macrophages—As mentioned earlier, a key event in atherogenesis is when monocytes differentiate into macrophages, leading to foam cell formation in the arterial intima. This will accelerate the development of arterial plaques. Scavenger receptors cause the uptake of oxLDL, which may contribute to foam cell transformation of macrophages and intracellular lipid accumulation. LPC is responsible for overexpressing the receptors, which will increase the numbers of foam cells in the atherosclerotic lesions.

LPC induces monocyte recruitment by up-regulating the expression of monocyte attractants to the arterial wall such as the counter-receptors for MCP-1, ICAM-1 and VCAM-1. Their overexpression will further accelerate atherogenesis. The LPC acyl-chain length is a significant component to stimulate IL-1 β production, recruit leukocytes, and activate endothelial cell functions and VSMCs mitogenesis, which are all involved in producing growth factors. The innate immune cells are major components in the vascular disease because they are involved in the early stages of lesion formation and accelerate its advancement from their derived molecules. For example, macrophages present in atherosclerotic lesions can further progress these lesions by secreting growth factors as well as proinflammatory cytokines (48).

6.2.5. LPC and oxLDL—LPC is a major component in oxLDL and plays a significant role in the promotion of atherosclerosis by oxLDL. The concentration of LPC is increased in atherosclerotic lesions (atheromata), suggesting the importance of the lysophospholipid's contribution to the inflammatory vascular disease. Many atherosclerotic lesions contain oxLDL, which is proven to stimulate endothelial cells, monocytes, macrophages and T-cells (49). OxLDL promotes atherogenesis by inducing cell death through oxidative stress (19). LPC plays a role in oxLDL-induced immune activation and has similar inducing effects as oxLDL in driving pro-inflammatory mechanisms such as recruiting monocytes into arteries (49).

6.3. LPS and AS

Lysophosphatidylserine (LPS) is a component of biological fluids and is present in human plasma but the concentration is currently unknown. The total concentration of LPS is detected to be about 0.13 mM in mouse serum and 10 mM in the aqueous humor of rabbits (5).

Only two articles can be found from PUBMED by using key words “lysophosphatidylserine” and “atherosclerosis” but they do not evaluate the effect of LPS on atherosclerosis. However, LPS has been reported to regulate many biological processes such as its characterized role in inflammation. LPS may potentially have a role in atherosclerosis because the treatment of LPS on mouse fibroblasts, human glioma cells and some human leukemia THP-1 cells lead to chemotactic migration.

6.4. LPE and AS

Lysophosphatidylethanolamine (LPE) is identified in the human serum and accumulates in the ischemic heart, but there have been no reports showing a significant association between LPE and atherosclerosis (50). LPE is demonstrated to be an important regulator of rat growth plate chondrocytes by TFG- β 1 direct and indirect activation, stored in the extracellular matrix.

6.5. LPI and AS

The biological activities of lysophosphatidylinositol (LPI) have not been studied as extensively as LPA and S1P. However, LPI produces a range of significant effects due to its origination in many cell types. The knowledge of LPI's potential functions has been limited until recently. The insight was gained by identifying LPI's orphan GPCR receptor, known as GPR55. GPR55 is expressed in adipose tissue, endothelial cells, inflammatory cells and cardiomyocytes, which can play a role in atherosclerosis (51). Since LPI activates GPR55, intracellular calcium concentrations increase, which, in effect, increase the release of insulin, mononuclear cell migration, arterial contraction, vasopressor response and platelet aggregation inhibition, and cell stimulation through endothelial electrical responses. LPI has a significant role in ECs because these cells have the ability to produce and release LPI, which may act in autocrine and paracrine manners. According to limited reports, LPI negatively regulates the migration of endothelial cells and induces the upregulation of adhesion molecules such as VCAM-1 and ICAM-1. Furthermore, when LPI induces the adhesion of circulatory inflammatory cells to the endothelium, the inflammatory cells stimulate the migration of VSMCs, which can potentially lead to atherogenesis (51). LPI also has an inhibitory effect on endothelium-dependent hyperpolarization induced by acetylcholine, a process mediating physiological vascular relaxation and dysfunction which is linked to the vascular disease. Therefore, LPI has a potential role in vascular inflammation by serving as a crucial contributor to endothelial dysfunction and development of atherosclerosis.

6.6. S1P and AS

S1P is a biologically activated, important lipid mediator, which is generated by sphingosine phosphorylation. S1P is bound to plasma proteins such as HDL and albumin, and is present in extracellular fluids. The major source of S1P in plasma is assumed to be red blood cells, activated platelets, vascular ECs, and other cells of hematopoietic origin. The actions of the lipid mediator are mediated by five GPCRs. SPR1 and SPR2 receptor subtypes have a significant role in macrophages and monocytes. These subtypes can have a vast effect in modulating cellular functions of atherogenic-contributing cells, such as cell proliferation, survival, migration and cell-to-cell adhesion in endothelial cells, smooth muscle cells,

macrophages and monocytes. However, S1P has both inhibitory and stimulatory properties, playing a dual role in anti- and pro-atherosclerosis development (28, 52).

6.6.1. The pro- and anti- effects of S1P on ECs—HDL is a known atheroprotective factor. S1P happens to be a component of HDL. Therefore, HDL-associated S1P displays anti-atherogenic properties in modulating endothelial cells. One of the first steps to atherogenesis is associated with EC barrier dysfunction and endothelial injuries (53). S1P promotes endothelial barrier integrity and suppresses the expression of ICAM-1 and VCAM-1 by down-regulating NF- κ B activity. The subtypes of S1P receptors have strong anti-inflammatory properties and mediate the response of immune cells by inducing anti-inflammatory cytokines, such as transforming growth factor β (TGF β), and pentraxin-3. In endothelial cells, adhesion decreases, the permeability of the barrier decreases and nitric oxide (NO) increases, which are critical factors in the regulation of vascular remodeling and angiogenesis (54).

However, there were also conflicting reports that show S1P's pro-atherogenic effects. Intracellular S1P induces the expression of adhesion molecules on endothelial cells, which promotes the attachment of leukocytes and initiates atherogenesis. S1P stimulates vascular EC migration, and induces EC proliferation through S1P receptors (55, 56).

6.6.2. The pro- and anti- effects of S1P on macrophages/monocytes—Although not many reports have solid evidence in S1P's role in macrophages and monocytes, some reports suggest that HDL-associated S1P inhibits pro-inflammatory effects of monocytes. SPR1 and SPR2 receptor subtypes are suggested to play conflicting roles in atherosclerotic progression depending on the site and level of expression and abundance of receptors; they may retard or promote the growth of atherosclerotic lesions (54). S1P may stimulate or inhibit the migration of monocytes and macrophages, leukocyte adhesions and pro- or anti-inflammatory cytokine production (28).

6.6.3. The pro- and anti- effects of S1P on VSMCs—As previously stated, one of the hallmarks of atherosclerotic progression is the phenotype of VSMCs switched from a differentiated contractile state to a de-differentiated synthetic state. S1P was shown to have many anti-atherosclerotic effects on VSMCs. Attributed with HDL, S1P may modulate HDL's inhibition of SMC migration, and mediate the activity of vasoactive components that target VSMCs. S1P is also reported to subdue the thrombin-induced pro-inflammatory activation of VSMCs (54). However, the opposing reports indicate that S1P has pro-atherogenic stimulatory properties that mediate VSMCs proliferation, migration and vasoconstriction (57).

6.6.4. The pro- and anti- effects of S1P on T-cells—S1P is also a double agent in the lipid-driven inflammatory disease when working in T-lymphocytes. S1P controls T-cell trafficking by regulating the distribution, activation, and proliferation of T-cells. The anti-atherogenic effects on T-cells are as follows: *first*, attenuate the anti-inflammatory phenotype of T-cells, *second*, decrease T-cell growth, and *third*, increase or decrease secretion of specific anti-inflammatory cytokines (54). The pro-atherogenic effects of S1P are assumed to act through a S1P intracellular signaling mechanism. Some reports showed

that S1P can prevent apoptosis in T-cells and leukocytes (58). This suggests that recruited T-cells and leukocytes can survive in the intima of the vascular wall and promote the release of inflammatory cytokines, thereby facilitating atherosclerotic development.

6.7. SPC and AS

SPC is a component of HDL particles, and is involved in many physiological responses such as cell migration, growth, proliferation, inhibition, and wound healing. It acts as a lipid mediator and is involved in intracellular signaling components linked with atherosclerosis, although it has not been studied in detail. SPC may have both pro- and anti-atherosclerotic properties. There are studies that show SPC can prevent the induced expression of endothelial adhesion molecules such as E-selectin, ICAM-1 and VCAM-1. However, it may also promote pro-atherogenic cells to enhance ICAM-1 expression and CCL2 production (59). Although it still remains poorly defined, SPC can induce the differentiation of stem cells to vascular smooth muscle cells through GPCR activation. SPC was also found to promote VSMC migration and contraction as well as the migration and dysfunction of endothelial cells. These reports suggest that SPC have some role in atherosclerosis and vascular inflammatory disease (60).

7. LPLS AND VASCULAR METABOLIC DISEASE

Lysophospholipids are involved in many vascular metabolic diseases due to their various effects on GPCR signaling pathways throughout the body. LPA and S1P are the two most extensively-studied lysophospholipids and are shown to be involved in the pathogenesis of many diseases (61). Although there are some beneficial effects of the lysophospholipids such as cardioprotection, their detrimental effects are also well reported. S1P and LPA can stimulate platelet aggression, which will enhance ischemia in acute coronary syndromes and myocardial infarction. They cause pro-inflammatory response to vascular injury and vasoconstriction of coronary, renal, and cerebral arteries, which suggests its role in myocardial hypertrophy and thrombus formation (62).

LPA is reported to be a bio-active mediator of many cardiovascular diseases such as acute thrombosis, platelet function in atherogenesis, vascular injury responses, neointima hyperplasia, and hypertension (61). LPA, via IL-6 secretions from VSMCs, is predictive of future coronary artery disease and is a regulator of vascular disease and inflammation (35). LPA triggers the inflammatory response in various vascular cells, activates human platelets, regulates vascular tone, and modulates the phenotype of SMCs. VSMCs have extensive phenotypic plasticity because it can switch phenotypes to adapt to the fluctuating environmental cues/signals and extracellular signals. This occurs during the development and progression of vascular diseases including atherosclerosis, asthma, hypertension and cancer (63, 64). LPA is suggested to be a modulator of VSMC plasticity as these cells undergo phenotypic modulation to distinct phenotypes within atherosclerotic lesions (63).

LPA is a potent bioactive lipid, which is present in the lipid-rich core of atherosclerotic plaques because of its heavy involvement in the development of atherosclerosis. Not only does LPA modulate VSMC phenotype, but it also modifies endothelial permeability and promotes EC migration, which are crucial steps in atherogenesis (61). Elevated levels of

LPA can worsen atheromatous lesions, through the activation of the early growth response gene-1 (Egr-1), an important zinc finger transcription factor in regulating many genes involved in vascular diseases (65). Egr-1 plays a crucial regulatory role in the pathogenesis of the cardiovascular system including myocardial hypertrophy, ischemia, angiogenesis, and atherosclerosis. LPA induces Egr-1 synthesis in VSMCs through the signaling pathway of LPA₁ receptor and the PKC-activated MAPKs including extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) (66).

Furthermore, LPA is a major bioactive lipid component of oxLDL. oxLDL is a cardiovascular risk factor (67). Another important component of oxLDL is LPC. LPC is an important factor in atherosclerosis because it induces monocyte migration, an early step in atherosclerotic development. According to reports, LPC activates kinase PKD in vascular cells, such as in platelets, endothelial cells, and vascular cells, suggesting PKD-mediated signaling pathways regulate myocardial contraction, hypertrophy, and remodeling. The kinase PKD has possible roles in atherosclerotic development and other inflammatory diseases (68).

Aside from the pathogenesis of vascular diseases, there are numerous beneficial effects that S1P exhibits for normal vascular development and its role in cardioprotection. It plays a protective role against acute myocardial ischemia because it accelerates neovascularization and blood flow recovery in ischemic limbs, which is useful for angiogenic therapy (23). It protects against apoptosis in cardiac myocytes and enhances cell survival by acting as a survival factor for HUVECs and cardiac fibroblasts (62). S1P is involved in embryonic vascular maturations, regulation of angiogenesis and vascular integrity, coronary perfusion possibly through coronary artery smooth muscle contraction, ischemic preconditioning and protection against ischemia and myocardial remodeling (23).

Due to the wide range and distribution of lysophospholipids and its signaling pathways throughout the body, LPA, LPC and S1P play a significant role in promoting and inhibiting the pathogenesis of vascular metabolic diseases.

8. CONCLUSION

LPLs, generated by phospholipase enzymes on membrane PLs and SLs, are small bioactive lipid mediators characterized by a single carbon chain and polar head group. Lysophospholipids act as intercellular signaling molecules by activating specific GPCRs as well as intracellular signal transduction. As extracellular mediators, their lysophospholipid signaling pathways exert many diverse cellular responses. Their receptors are expressed in a wide range of various tissues and cell types including endothelial cells, fibroblasts, vascular smooth muscle cells, macrophages and monocytes, T-lymphocytes, platelets and dendritic cells. Lysophospholipids play a role in cellular processes including cell adhesion, motility, proliferation, cell survival and apoptosis, cytoskeletal changes, phenotype modulation, angiogenesis and process retraction. These cell responses are required for normal system development and function but they can also have specific roles in the pathogenesis of diseases such as cancer, Alzheimer's disease, hypertension, and atherosclerosis. Atherosclerosis is a lethal chronic arterial disease that involves the buildup of plaques within

the arteries, which can cause plaque rupture, myocardial infarction and strokes. LPA and S1P are two lysophospholipids studied in great detail in various cell types and in their involvement in the development and progression of atherosclerosis. Other lysophospholipids including LPC, LPS, LPI, and SPC are also involved in atherogenesis. LPE has not been recognized as a significant factor in the disease. Overall, the derivatives of phospholipids are crucial factors in atherogenesis because they have significant roles in the pathogenesis of the vascular diseases including changing VSMC phenotype, the hallmark of progressive atherosclerosis. The biological functions and signaling pathways of lysophospholipids are of importance in many of the physiological and pathophysiological processes including abnormal development, atherosclerosis and many diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

LPLs	lysophospholipids
PLs	phospholipids
SLs	sphingolipids
LPA	lysophosphatidic acid
S1P	sphingosine 1-phosphate
GPCRs	G protein-coupled receptors
Rock	Rho-associated kinase
DAG	diacylglycerol
IP3	inositol 1,4,5-trisphosphate
MAPK	mitogen-activated protein kinase
AC	adenylate cyclase
PI3K	phosphoinositide-3-kinase
LPL-GPCRs	G protein-coupled LPLs receptors
EDGs	endothelial differentiation genes
PA	Phosphatidic acid
TAG	triacylglycerols
PC	phosphatidylcholine
PE	phosphatidylethanolamine

CDP-DAG	cytidine diphosphate diacylglycerol
PG	phosphatidylglycerol
PI	phosphatidylinositol
PS	phosphatidylserine
GCS	glucosylceramide synthase
PLA1	Phospholipase A1
PLA2	phospholipase A2
PLB	phospholipase B
LysoPLA1/2	lysophospholipase A1/2
PLC	phospholipase C
PLD	phospholipase D
sPLA2s	secreted PLA2s
cPLA2s	cytosolic PLA2s
iPLA2s	Ca ²⁺ -independent PLA2s
PAF-AHs	acetylhydrolases
AdPLA	adipose-specific PLA2
SPC	Sphingosylphosphorylcholine
SPE	sphingosylphosphoethanolamine
SPI	sphingosylphosphoinositol
AS	atherosclerosis
ECs	endothelial cells
VSMCs	vascular smooth muscle cells
DCs	dendritic cells
LDL	low density lipoprotein
oxLDL	oxidized LDL
VCAM-1	vascular cell adhesion molecule-1
ICAM-1	intercellular adhesion molecule-1
MCP	monocyte chemo-attractant protein
IL-1β	interleukin-1 β
CXCL1	chemokine (C-X-C motif) ligand 1
ROI	reactive oxidative intermediates
LPC	lysophosphatidylcholine

eNOS	endothelial nitric oxide synthase
PKC	protein kinase C
LPS	lysophosphatidylserine
LPE	lysophosphatidylethanolamine
LPI	lysophosphatidylinositol
TGFβ	transforming growth factor β
NO	nitric oxide
ERK	extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase

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Figure 1. Chemical structure of different species of glyceryl-based lysophospholipids (glyceryl-LPLs) and sphingosyl-based lysophospholipids (sphingosyl-LPLs)

Glyceryl-LPLs and sphingosyl-LPLs have either a glycerol or a sphingosine backbone, respectively. However, they are all characterized by having a single tail of carbon chain (R, fatty acid residue) and a phosphate group (S). A phosphate has 5 possible bases: Choline, Serine, Ethanolamine, Glycerol and Inositol. For LPA, the S should be just an H (hydrogen).

Figure 2. Lysophospholipid Receptor Signaling

Lysophospholipids are bioactive lipid derivatives commonly derived from cell membranes. The most important actions of lysophospholipids are mediated by 7-transmembrane G protein-coupled receptors (GPCRs) on the cell surface that now number at least 26. LPLs signals through its own GPCRs via four distinct classes of G proteins — G_s , $G_{q/11}$, G_i and $G_{12/13}$ — leading to activation of multiple downstream effector pathways, which lead to physiological and pathophysiological functions such as cell migration, proliferation, survival, cell-cell reaction, cytoskeletal remodelling, etc.

Figure 3. Chemical structure of different species of phospholipids and sphingolipids

Phospholipids are formed by a molecule of glycerol, by 2 fatty acids in position 1 and 2 (R_1 and R_2) and a phosphate having 5 possible bases (S): Choline, Serine, Ethanolamine, Glycerol and Inositol. Instead of glycerol, sphingolipids have a sphingosine backbone. Sphingomyelins are the only sphingolipids class and also phospholipids. With different phosphate groups (S), sphingomyelins can be termed as Ceramide-PC (with phosphocholine group), Ceramide-PE (with phosphoethanolamine group) and Ceramide-PI (with phosphoinositol group).

Figure 4. Pathway for biosynthesis of lysophospholipids

PA, Phosphatidic acid; DAG, sn-1,2-diacylglycerols; CDP-DAG, cytidine diphosphate diacylglycerol; TAG, triacylglycerols; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; S1P, sphingosine 1-phosphate; SPC, Sphingosylphosphorylcholine; SPE, sphingosylphosphoethanolamine; SPI, sphingosylphosphoinositol.

Figure 5. Several enzymes (phospholipases and phosphodiesterases) are responsible for generation and degradation of lysophospholipids

Phospholipases are a group of enzymes that hydrolyze phospholipids into fatty acids and other lipophilic molecules. Phospholipases of the A type (PLA) remove one of the two fatty acids, producing a lysophospholipid; PLAs are subdivided into PLA₁, which cleave phospholipids at the sn-1 ester bond and PLA₂, which cleave at the sn-2 bond. Lysophospholipases (LysoPLA or PLB) remove the remaining fatty acid. The phosphodiesterases are represented by phospholipase C (PLC) and phospholipase D (PLD).

Table 1

Identified G-protein coupled receptors for lysophospholipids.

G Protein Receptors	Aliases	Ligands (lysophospholipids)	Gene Symbols (human)
LPA ₁	EDG2, GRP26, VZG-1	LPA	LPAR1
LPA ₂	EDG4	LPA	LPAR2
LPA ₃	EDG7	LPA	LPAR3
LPA ₄	P2Y9, GPR23, P2Y5-LIKE	LPA	LPAR4
LPA ₅	GPR92, GPR93	LPA	LPAR5
LPA ₆	P2Y5, LAH3	LPA	LPAR6
S1P ₁	EDG1, LPB1, CD363	S1P, SPC	S1PR1
S1P ₂	EDG5, GPCR13, LPB2, AGR16	S1P, SPC	S1PR2
S1P ₃	EDG3, LPB3	S1P, SPC	S1PR3
S1P ₄	EDG6, LPC1	S1P, SPC	S1PR4
S1P ₅	EDG8, LPB4, SPPR-1, SPPR-2	S1P, SPC	S1PR5
GPR3	ACCA	S1P	GPR3
GPR4	-	SPC, LPC	GPR4
GPR6	-	S1P	GPR6
GPR12	GPCR12, GPCR21	S1P, SPC	GPR12
GPR34	-	LPS	GPR34
GPR35	-	LPA	GPR35
GPR45	PSP24	S1P	GPR45
GPR55	-	LPI	GPR55
GPR65	TDAG8	SPC, LPC	GPR65
GPR68	OGR1, GPR12A	SPC, LPC	GPR68
GPR87	GPR95	LPA	GPR87
GPR119	GPCR2	LPC, LPE, LPI	GPR119
GPR132	G2A	LPC, SPC, LPS, LPE	GPR132
P2Y10	P2Y10	LPA, S1P	P2RY10
PTAFR	PAFR	LPC, Lyso-PAF	PTAFR

LPA: lysophosphatidic acid; S1P: sphingosine 1-phosphate; GPR/GPCR: G protein-coupled receptor; SPC: Sphingosylphosphorylcholine; LPC: lysophosphatidylcholine; LPS: lysophosphatidylserine; LPE: lysophosphatidylethanolamine; LPI: lysophosphatidylinositol; Lyso-PAF: Lyso-platelet-activating factor; PTAFR: platelet-activating factor receptor; EDG: endothelial differentiation gene; P2Y: purinergic G protein-coupled receptors