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Lysophosphatidic acid effects on atherosclerosis and thrombosis

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Abstract

Lysophosphatidic acid (LPA) has been found to accumulate in high concentrations in atherosclerotic lesions. LPA is a bioactive phospholipid produced by activated platelets and formed during the oxidation of LDL. Accumulating evidence suggests that this lipid mediator may serve as an important risk factor for development of atherosclerosis and thrombosis. The role of LPA in atherogenesis is supported by the evidence that LPA: stimulates endothelial cells to produce adhesion molecules and chemoattractants; induces smooth muscle cells to produce inflammatory cytokines; stimulates smooth muscle cell dedifferentiation, proliferation, and migration; increases monocyte migration and decreases monocyte-derived cell emigration from the vessel wall; induces hypertension and vascular neointimal formation *in vivo*; and promotes plaque progression in a mouse atherosclerosis model. The role of LPA in thrombogenesis is supported by the evidence that LPA markedly induces the aggregation of platelets and the expression of tissue factor, which is the principal initiator of blood coagulation. Recent experimental data indicate that LPA is produced by specific enzymes and that LPA binds to and activates multiple G-protein-coupled receptors, leading to intracellular signaling. Therapeutics targeting LPA biosynthesis, metabolism and signaling pathways could be viable for prevention and treatment of atherosclerosis and thrombosis.

Keywords

atherosclerosis; lysophosphatidic acid; lysophosphatidic acid receptor; lysophospholipase D; thrombosis; vascular disease and therapeutics; vascular lesion

Lysophosphatidic acid (LPA; 1-acyl 2-hydroxyl glycerol 3-phosphate) is one of the simplest phospholipids; however, the understanding of its biological function in health and disease is ever growing. The earliest report regarding LPA's physiological action was by Vogt in 1963 [1]. In this article, it was demonstrated, using isolated strips of rabbit duodenum, that LPA has smooth muscle stimulating action. In 1978, Tokumura and colleagues used animal models to provide the first evidence that LPA has dramatic physiological activity *in vivo* [2]. This study demonstrated that intravenous injection of LPA modulates blood pressure in rodents [2], revealing that LPA affects the function of blood vessels. LPA alteration of cerebrovascular reactivity was later reported in piglets [3], and the discovery that LPA stimulates platelet aggregation was among the first recognition that LPA functions in

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thrombotic vascular disease [4,5]. Although phosphatidic acid (PA) or LPA was suggested to be the vasoactive component in plasma incubated for a long period of time [4], the identification of LPA, but not PA, to be this active vasoactive component was reported by Tokumura's group in 1986 [6]. Also promoting today's prosperous LPA research in various physiology and disease areas are the initial discovery by Moolenaar *et al.* that LPA functions as an extracellular agonist, which triggers cellular mitogenesis via G-protein-mediated intracellular signaling pathways [7–9]; the identification of LPA's cognate plasma membrane receptors by Chun's group and others [10–18]; and the success of genetic knockout of these LPA receptors [19–21]. Since accumulating evidence from epidemiologic studies suggests that an elevated level of oxidized LDL (oxLDL) is one of the major risk factors of cardiovascular disease [22,23] and has a key atherogenic impact [24–27], the discovery made by Siess and colleagues in 1999 that LPA is a bioactive constituent in mildly oxLDL and atherosclerotic lesions has increased interest in the study of a role of LPA in atherosclerosis and thrombosis.

LPA synthesis & degradation in the vascular system

Lysophosphatidic acid is a simple lipid molecule with a glycerol backbone, a phosphate residue, and an ester-linked fatty acid with a variable length carbon chain that is either saturated or unsaturated (Figure 1). Several investigators in the 1960s and 1970s suggested serum to be the biological source of LPA [1,4]. Tokumura's group first successfully identified LPA in long-incubated rat plasma using 2D, thin-layer chromatography and mass spectrometry technologies [6]. The authors hypothesized that an enzyme called lysophospholipase D (lysoPLD) mediates the conversion of a high concentration of lysophosphatidylcholine (LPC; several hundred micromolar) to LPA in incubated plasma. However, the identity of this hypothesized enzyme remained unknown for a long time. It was not until 2002 that this long-proposed enzyme was finally purified and identified by Aoki's group [28] and Tokumura's group [29]. Now lysoPLD is considered the major LPA-generating enzyme. Interestingly, the sequence of this enzyme was determined to be identical to autotaxin, a tumor cell motility-stimulating factor [30,31]. Regarding LPA production and the function of lysoPLD, readers are referred to read two recent comprehensive reviews [32,33].

Lysophospholipase D mediation of LPA production in circulating blood is supported by evidence that heterozygous lysoPLD^{+/-} mice have about half the normal circulating levels of both lysoPLD and LPA [34,35] and transgenic overexpression of human lysoPLD in mice increases levels of plasma lysoPLD and LPA [36]. The recent results that lysoPLD can bind to integrins of lymphocytes [37] and platelets [38] suggest localized production of LPA on these cell surfaces via digestion of LPC. Degradation of plasma levels of LPA can be regulated by LPA degradation enzymes, lipid phosphate phosphatases, which dephosphorylate LPA to become monoacylglycerol [39,40]. LPA acylation by LPA acyltransferase to become phosphatidate may also reduce plasma levels of LPA [39,40].

Lysophosphatidic acid production and release from platelet activation has also been reported [41–44]. In this case, LPA is converted from PA by phospholipase A₁/phospholipase A₂ activities. PA is produced from phospholipid and diacylglycerol by phospholipase D and diacylglycerol kinase, respectively [32].

Lysophosphatidic acid formation during oxidation of LDL and accumulation in high concentrations in human atherosclerotic lesions, particularly in their lipid-rich cores (10–49 μ M), has been revealed by Siess *et al.* [45]. The mean level of LPA increases 13-fold in atheromatous plaques compared with normal arterial tissue [45]. Similar results have been reported recently in a mouse model of atherosclerosis induced in carotid arteries of LDL

receptor-deficient mice; LPA content increases over 40-fold in mouse atherosclerotic carotid artery lesions (8 weeks of treatment) compared with control animals [46]. One possible mechanism responsible for the accumulation of LPA in atherosclerotic lesions may be increased expression of the LPA-generating enzymes cytoplasmic phospholipase A₂ and calcium-independent PLA₂ and the decrease of LPA acyltransferase- α in atherosclerotic lesions [46].

The aforementioned LPA production routes and sources in vascular systems imply possible functions of LPA in the development of vascular diseases, especially atherosclerosis and thrombosis.

Role of LPA in atherosclerosis & thrombosis

Atherosclerosis, one type of vascular disease involving hardening of the arteries, is a major cause of heart attacks and stroke. Atherosclerosis is characterized by the accumulation of lipids, macrophages, smooth muscle cells (SMCs), T lymphocytes and fibrous tissue in the inner-most layer of large- and medium-sized arteries. The development of atherosclerosis has been comprehensively reviewed [22,47–49]. One of the initial steps of atherosclerosis includes endothelial cell (EC) activation, which produces adhesion molecules in the EC plasma membrane. These adhesion selectin molecules initiate the tethering and rolling of leukocytes to the endothelium. Firm adhesion and spreading of leukocytes onto endothelium requires interaction between leukocyte β 1- and β 2-integrins and endothelial immunoglobulin superfamily members, such as VCAM-1 and ICAM-1. Then, proinflammatory chemokines produced in the intima attract leukocytes that have adhered on the endothelium. Intermediate steps of atherosclerotic lesion development include foam cell formation and the accumulation of T lymphocytes and monocyte-derived dendritic cells (DCs) joining macrophages in the intima. Cytokines and growth factors secreted from these leukocytes and resident vascular wall cells, along with bioactive lipids produced in the intima, promote more leukocytes into the intima and induce SMC migration and proliferation in the intima. SMCs form a fibrous matrix and wall the neointimal plaque. Ultimately, inflammatory mediators can inhibit collagen synthesis, evoke the expression of collagenases, and induce tissue factor (TF) expression in the plaques. Therefore, when plaques rupture, TF produced in the plaque will activate factor VII in the bloodstream, leading to the activation of the blood coagulation pathway, resulting in thrombin formation, which then leads to fibrin production and cross-linking. The fibrin deposition together with platelet activation and aggregation build up thrombi that cause most acute complications of atherosclerosis–thrombosis. The activation, inflammation, migration and proliferation of adventitia fibroblasts may also contribute to the development of atherosclerosis [50–52].

Accumulating evidence suggests that LPA possibly participates in most of the processes involved in atherogenesis and thrombosis.

LPA's effect on vascular ECs

The effects of LPA on vascular ECs include:

- LPA induces adhesion molecule expression in ECs. LPA activates ECs to produce adhesion molecules that include E-selectin, VCAM-1 and ICAM-1 on the cell surface [53–55]. LPA receptor 1 (LPA₁) is responsible for LPA-induced ICAM-1 expression [56]. LPA induction of the expression of ICAM-1 and VCAM-1 in ECs is mediated through the Rho kinase-2-triggered NF- κ B pathway [57]. These adhesion molecules expressed on the surface of ECs can help tether, roll and spread monocytes and lymphocytes onto the endothelium in the vascular wall, contributing to the initiation of atherogenesis;

- LPA induces inflammatory cytokine secretion from ECs. LPA stimulates ECs to generate proinflammatory cytokines and chemoattractant cytokines (chemokines) such as IL-1 β , IL-8 and monocyte chemoattractant protein (MCP)-1 [54,58]. Furthermore, ECs stimulated by LPA have been demonstrated to enhance monocytic cell recruitment to the surface of ECs via LPA-induced IL-8 and MCP-1 secretion from ECs [58]. LPA-induced secretion of IL-8 and MCP-1 from ECs, as well as chemotaxis of monocytic cells, is mediated by EC surface LPA₁ and LPA₃ via an IL-1-dependent pathway [56,58]. It is well established that IL-1 β promotes the development of atherosclerosis [59]; IL-8 and MCP-1 secreted from ECs trigger blood leukocytes to attach and spread or transmigrate on endothelium [22,60–63]. In analyzing the secretome of ECs activated by LPA, it was found that LPA induces secretion of pentraxin-3, which belongs to the pentraxin family of acute-phase proteins, and that the secreted pentraxin-3 has chemotactic activity on human THP-1 monocytic cells [64], suggesting that LPA-induced pentraxin-3 from ECs may also contribute to the development of atherosclerosis;
- LPA's effect on EC permeability is controversial. Experimental results regarding the effect of LPA on EC permeability have not been consistent. Both increases and decreases of endothelial barrier function have been reported in response to LPA. For example, there are reports demonstrating that LPA induces a prolonged decrease in endothelial barrier function and causes endothelial detachment in human umbilical vein ECs [65]. In isolated rat mesenteric venules, LPA treatment caused an increase in hydraulic conductivity [66]. In addition, LPA-increased permeability of pig brain capillary ECs and cerebral endothelium in culture has also been reported [67,68]. However, there are also reports demonstrating that LPA protects vascular integrity and decreases permeability of bovine aortic ECs [69], stabilizes monolayer barrier function of bovine pulmonary artery ECs in contrast to thrombin's effect [70], and decreases monolayer permeability of human umbilical vein EC line EA.hy926 [71]. The discrepancies in these observations may be due to EC heterogeneity [72] (differences between species, macrovascular ECs vs micro-vascular ECs and artery ECs vs vein ECs), differences in cell culture and experimental conditions, as well as methodologies of detection of barrier function. Impairment of endothelial barrier function contributes to vascular inflammation and atherosclerosis;
- LPA stimulates angiogenesis. Angiogenesis involves the proliferation and migration of ECs to form newly sprouting blood vessels from existing vasculature, an essential process during development. However, angiogenesis has also been recognized as an important process for the progression of atherosclerotic plaques [73–77]. Intraplaque angiogenesis is not only related to plaque growth, but also to plaque instability [78,79]. LPA induces EC migration and proliferation *in vitro* [80,81] and enhances expression of matrix metalloproteinase-2 ECs [82]. In addition, LPA induces EC capillary tube formation *in vitro* and *in vivo* [83,84] and induces angiogenesis *in vivo* in a vessel sprouting assay for new vessel formation in chicken chorioallantoic membrane [85]. These effects exerted by LPA may contribute to atherosclerotic plaque formation and disability.

LPA's effect on vascular SMCs

The effects of LPA on vascular SMCs include:

- LPA induces SMC phenotype changes. Phenotype modulation of SMCs is considered an essential event in the pathogenesis of atherosclerosis [86–90]. Evidence suggests that intimal SMCs differ significantly from medial SMCs and that they may have unique atherogenic properties that make them fertile ground for the initiation of plaques [91]. Unsaturated LPA induces rat SMC phenotype to change from 'contractile' to 'synthetic' [92]. This LPA-induced SMC phenotype change is mediated through the coordinated activation of extra-cellular signal-regulated kinase and p38 mitogen-activated protein kinase [92]. Phenotype modulation of cultured SMCs can be divided into early stage (within hours) and late stage (within days). However, a recent article reports that the early stage of phenotype modulation (within hours) is independent of LPA stimulation [93].
- LPA induces SMC migration and proliferation. During atherogenesis, vascular SMCs migrate to populate the intima, either from the media [94] or from the circulation via migration of CD34⁺ hematopoietic progenitor cells, giving rise to smooth muscle progenitor cells [95]. In animal models of vascular injury, intimal and medial thickening is thought to be attributable to vascular SMC migration as well as proliferation from media to intima [96–98]. Evidence from studies has demonstrated that SMCs are dominant cell types in atherosclerotic lesions [99–101]. Understanding the risk factors that induce SMC migration and proliferation in atherosclerotic lesions is of great clinic importance. For example, LPA induction of primary human, calf and mouse artery SMC migration and proliferation have been reported [102–106]. Besides LPA, phospholipids, such as LPC, 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine, and 4-hydroxynonenal are also important bioactive components of oxLDL [107,108] and are known to play a significant role in atherogenesis [109–113]. In comparison with the other lipid contents in oxLDL, our results indicate that LPA is the most bioactive chemotactic and mitogenic to SMCs (Figure 2). LPA induces reactive oxygen species (ROS) in SMCs [114–116], which mediate LPA-induced SMC proliferation and chemokine expression [116]. These effects of LPA could be responsible for promoting SMC accumulation in the atherosclerotic lesions.
- LPA induces secretion of inflammatory cytokines and chemokines from vascular SMCs. Our recent cytokine antibody array results demonstrate that LPA prominently induces the secretion of IL-6 and MCP-1 from human aortic SMCs [117]. These data reveal that LPA₁ mediates LPA-induced IL-6 secretion and that PKC-mediated p38 α MAPK is responsible for the IL-6 secretion. LPA-stimulated MCP-1 secretion can be blocked by pitavastatin, suggesting that LPA-induced MCP-1 expression in SMCs is mediated by Rac-1 regulated, NADPH oxidase-dependent ROS generation [116]. In light of the critical role of MCP-1 in the initiation of atherosclerosis [118] and the role of IL-6 in vascular inflammation, it is possible that LPA-induced MCP-1 in the intima in SMCs and other inflammatory cells can direct the migration and diapedesis of adherent monocytes and T lymphocytes into the arterial intima promoting atherogenesis, and that LPA-induced IL-6 travels to the liver, where it elicits the acute-phase response, resulting in the release of C-reactive protein, fibrinogen and plasminogen activator inhibitor-1. All these inflammatory markers and mediators, released at different stages in the pathobiology of atherothrombosis, can enter the circulation to worsen atherosclerotic lesions.
- LPA induces coagulation initiator TF expression in SMCs. Our early results revealed that LPA markedly induces the expression of TF in SMCs [119]. TF,

also known as tissue thromboplastin or coagulation factor III, a transmembrane glycoprotein, is the principal initiator of blood coagulation, regulates hemostasis, and plays an important role in arterial thrombosis [120–123]. The critical role of SMC TF in macrovascular thrombosis is highlighted in a recent article, in which an SMC-specific, TF-deficient mouse model was used [124]. It is possible that LPA-induced overexpression of TF in SMCs in lesions on the vascular wall contributes to vascular occlusion and thrombosis. Our subsequent study demonstrated that LPA highly induces transcription factor Egr-1 expression [125], which was shown to control TF expression in various cell types including SMCs [126,127]. Therefore, it is likely that LPA induction of Egr-1 contributes to TF induction, which in turn, promotes thrombus formation, leading to thrombosis.

LPA's effect on fibroblasts

Lysophosphatidic acid induction of fibroblast cell proliferation was revealed by one of the early reports regarding LPA's effect on cellular functions [7]. LPA also induces fibroblast cell migration [128]. Although as discussed above, it is well recognized that vascular SMC migration and proliferation are essential components of atherogenesis, recent data also indicate that excessive proliferation and migration of adventitial fibroblasts play an important role in the pathobiology of atherosclerosis and restenosis [51,52]. Therefore, LPA induction of proliferation and migration of fibroblast cells may contribute to the development of atherosclerosis.

LPA's effect on monocytes & macrophages

It has been demonstrated that LPA modulates monocytic cell migration directly and indirectly via its induced secretion of IL-8 and MCP-1 from ECs [129,130]. The experimental results from Fueller *et al.* indicate receptor-mediated activation of monocytes by LPA [131]. Monocyte migration from the bloodstream into the subendothelial space of the vascular wall is one of the initial steps leading to the development of atherosclerosis. Therefore, evidence of LPA mediation of monocyte migration implies that LPA could promote early stages of atherogenesis. LPA has also been reported to significantly increase both DNA synthesis and production of reactive oxygen intermediates (ROI), as well as arachidonic acid and prostaglandin E2 release in and from monocytic cells [132]. Studies have demonstrated that ROI generation by the monocyte/macrophage system at inflamed sites can affect inflammatory processes [133,134]. ROI mediates cyclooxygenase-2 induction during monocyte to macrophage differentiation [135]. Arachidonic acid release from monocytes is involved in the early stages of monocyte activation [136] and prostaglandin E2 release by blood monocytes is related to subclinical atherosclerosis [137]. Therefore, LPA modulates monocyte proinflammatory activation to produce ROI and release arachidonic acid and prostaglandin E2, which may worsen vascular lesions. The observations that LPA impairs the capacity of monocyte-derived cells to emigrate from the vessel wall, and that LPA causes maximal neutral lipid accumulation in most monocyte-derived cells [138], support a notion that LPA favors monocyte retention and foam cell formation in subendothelium, promoting the progression of atherosclerosis. Understanding LPA's effect on monocytes and monocyte-derived cells, may help in developing promising new therapeutic strategies in managing monocyte migration, inflammation, retention and foam cell accumulation. In an early report, it was demonstrated that LPA also protects macrophages from apoptosis [139]. It is well known that in the late stages of atherosclerosis, macrophage apoptosis promotes atherosclerotic plaque rupture; however, in the early stages of atherosclerosis, protection of macrophages from apoptosis by LPA may enhance lesion cellularity and foam cell formation, resulting in plaque formation. LPA has been demonstrated to induce oxLDL uptake by murine macrophages, leading to foam cell

formation [140]. Therefore, LPA could promote atherosclerotic lesion formation by protecting macrophage apoptosis and promoting oxLDL uptake by macrophages. Furthermore, it has been reported that LPA enhances lipopolysaccharide-induced CXC chemokine ligand 16 expression in macrophages, and that the secreted ligand contributes to T-lymphocyte migration [141]. LPA induces inflammatory cytokine IL-1 β expression, which is dependent on ROS mediation in macrophages [142]. Secreted cytokine IL-1 β from macrophages would likely promote ECs to produce adhesion molecules, such as ICAM-1 and ECAM-1 [143]. IL-1 β also enhances growth factor-induced SMC proliferation [144]. These effects of LPA on macrophages may positively enhance atherogenesis.

LPA's effect on DCs

The influence of LPA on human monocyte-derived DCs was first reported by Panther *et al.* [145]. These authors differentiated CD14 precursors into immature DCs with IL-4 plus granulocyte-macrophage colony-stimulating factor, and then studied the influence of LPA before and after maturation with high-dose lipopolysaccharide. They found that the influence of LPA is markedly dependent on the maturation status of DCs. LPA stimulates calcium flux, actin polymerization, and chemotaxis of immature DCs [145] and induces maturing DCs to secrete IL-6 and IL-8 [146]. These results indicate that LPA enhances DC maturation [147]. LPA₃ mediates chemotaxis of immature murine DCs to unsaturated LPA [148]. These functions of LPA could help to recruit immature DCs into subendothelium and enhance DC functions in arterial and peripheral target sites.

LPA's effect on T lymphocytes

Lysophosphatidic acid induction of lymphocyte (Jurkat T cell) proliferation and IL-2 production upon activation with phorbol esters was first reported by Xu *et al.* [149]. It has been demonstrated that LPA-induced IL-2 secretion on mitogen-activated T cells is mediated by LPA₁ [150], but unstimulated human CD4⁺ T cells predominately express LPA₂. After activation with a mitogenic lectin, the pattern of expression of LPA receptors changes rapidly and significantly [150]. The level of LPA₂ in the T-helper subset decreases by approximately 50%. LPA protects human T-lymphoblastoma cells from apoptosis via LPA₁ and LPA₂ [151]. LPA also stimulates lymphocyte migration. Studies have demonstrated that LPA stimulates Jurkat T-cell migration and secretion of matrix metalloproteinases when LPA₂ is overexpressed in these cells, whereas in LPA₁-dominant Jurkat T cells, LPA does not evoke migration, suppress chemokine-elicited migration, or enhance IL-2 generation [152]. LPA under submaximal activation conditions preferentially enhances the secretion of the T-cell effector cytokine IL-13, which involves transcriptional induction of specific IL-13 promoter sequences [153].

These results suggest that the effects of LPA on promoting T-lymphocyte proliferation and migration, preventing T-lymphocyte apoptosis, and inducing inflammatory cytokine expression may facilitate atherogenesis. LPA induction of matrix metalloproteinase secretion from T lymphocytes may contribute to destabilization of plaques, promoting plaque rupture, which, in turn, contributes to atherothrombosis.

LPA's effect on platelets

Among the now well-known evidence that various types of cells are responsive to LPA stimulation, the platelet's activation, shape change and aggregation stimulated by LPA are the earliest discoveries of LPA's effects on isolated cells [4,5,154]. LPA, at concentrations slightly above plasma levels, induces platelet shape change and aggregation in whole blood [155]. LPA is a major lipid component in oxLDL that induces platelet shape change [45]. Furthermore, LPA induces assembly of fibronectin by adherent platelets [156], and fibronectin deposition and matrix formation on the surface of activated platelets may

enhance the formation and stability of thrombi. LPA also stimulates platelet-monocyte aggregation in whole blood [155], which is considered an early marker of acute myocardial infarction. It has been reported that platelet aggregation induced by components of the atherosclerotic plaque lipid-rich core can be inhibited by LPA receptor antagonists [157], indicating that LPA is a functional component in atherosclerotic plaques causing platelet aggregation. Therefore, it is likely that following plaque rupture or erosion, exposure of LPA in the lipid-rich core or production of LPA at sites of platelet activation may trigger platelet aggregation, which contributes to acute thrombosis. LPA₅ in particular has been reported to mediate LPA signals leading to platelet activation and aggregation [158].

Although LPA stimulates platelet aggregation in the majority of healthy human beings, it should be noted that aggregation of human platelets in response to LPA is far from homogenous [159,160]. It was reported that in 3% of healthy humans (data from more than 30 healthy donors), platelets demonstrated a much lower aggregatory response to highly unsaturated LPA, but responded to alkyl-glycerophosphate (AGP; the analog of acyl-LPA) well [159]. In less than 7% of those same 30 healthy humans, platelets did not respond to alkyl-LPA, but responded to LPA normally. A recent article reported that about 20% of healthy human platelets did not respond to LPA (data from 45 healthy donors) [160]. In addition, LPA does not stimulate mouse platelet aggregation [38], indicating that distinct regulatory pathways may contribute to platelet aggregation in different species. Interestingly, among the 70 patients with stable coronary artery disease, 99% of patients were identified as LPA responders, suggesting that LPA may be a pathophysiologically relevant mediator of cardiovascular disease in humans and a viable target for novel antithrombotic strategies.

Taken together, LPA has potent and numerous effects on vascular wall cells and blood cells *in vitro*. A summary of these effects of LPA is illustrated in Figure 3, implying a potential role of LPA in the development of atherosclerosis and thrombosis.

LPA affects vascular wall function *in vivo*

As described above, potent and numerous effects of LPA on vascular wall cells and blood cells *in vitro* include proliferation, differentiation, migration, inflammation, survival, aggregation, contractile activity, lipid uptake and coagulation. Therefore, these effects of LPA on vascular wall function *in vivo* were highly expected. Indeed, intravenous injection of LPA elevates arterial blood pressure in rodents [161] and local application causes cerebral vasoconstriction in pigs [3]. Furthermore, local infusion of LPA in rat and mouse common carotid arteries induces vascular remodeling by stimulating neointimal formation [162,163]. As demonstrated in Figure 4, infusion of LPA into rat carotid artery causes marked neointimal formation. LPA-induced hypertension and neointimal formation in the vascular wall likely contribute to the development of atherosclerosis. However, to date, the mechanism by which LPA triggers hypertension and neointimal formation has not been made clear. It has been demonstrated that the LPA ether analog AGP stimulates vascular neointimal formation via peroxi-some proliferator-activated receptor- γ but not the LPA plasma membrane receptors [164]. However, another article demonstrated that the antagonist Ki16425 of LPA receptors LPA₁ and LPA₃ blocked AGP-induced neointimal formation in mouse carotid arteries, suggesting that LPA₁ and LPA₃ mediate AGP-induced neointimal formation [165]. The discrepancy in these results may be due to differences in experimental procedures, animal models used, or nonspecific effects of the chemical antagonists applied. Furthermore, it has been demonstrated that transient increases in blood pressure following intravenous administration of LPA to mice is independent of either LPA₁ or LPA₂ [105]. Interestingly, a direct link of LPA's effect on the development of atherosclerosis has been demonstrated in a recent study, which shows that local and systemic application of LPA accelerates the progression of atherosclerosis in atherosclerotic

mouse model-ApoE knockout mice, suggesting that LPA-induced C-X-C motif ligand (CXCL)-1 expression on the surface of endothelium might play a role in monocyte adhesion leading to atherogenesis [166]. In addition to the results obtained from the direct intravenous administration of LPA, it has also been demonstrated that ligation injury-induced neointimal hyperplasia in mouse carotid arteries is reduced in LPA₁/LPA₂ deficient mice, suggesting that LPA₁ and LPA₂ partially regulate ligation injury-induced neointimal formation [105]; however, in another mouse model with wire-induced injury of the carotid artery, the pharmacological antagonist Ki16425 of LPA receptors LPA₁ and LPA₃ reduced wire injury-induced neointimal formation [165], suggesting that LPA₁/LPA₃ mediate wire injury-induced neointimal formation. Although the LPA receptor's roles in these different kinds of mechanical injury-mediated neointimal formation are inconsistent with each other, the interesting implication is obvious: that mechanical injury may cause accumulation of LPA in the injured vascular wall. Direct experimental evidence to support this hypothesis is necessary. If proven, LPA may also serve as a key mediator for restenosis, in addition to atherosclerosis.

Conclusion & future perspective

Lysophosphatidic acid concentrations are highly elevated in the lipid-rich core of human athero-sclerotic plaques, where LPA is likely derived from mildly oxLDL [45]. Many reported effects of oxLDL on vascular wall cells and blood cells may result from LPA. As reviewed above, a high concentration of LPA in the circulation system may be a risk factor for the development of atherosclerosis and thrombosis. LPA activates ECs to secrete chemoattractant cytokines to attract monocytes and lymphocytes toward the endothelium of vascular walls. LPA stimulates the production of adhesion molecules on the surface of ECs to tether and roll monocytes and lymphocytes to the endothelium. Adhesion and rolling of monocytes and lymphocytes to endothelium are initial steps of atherosclerosis. LPA induces SMC differentiation, proliferation, and migration, which are important features of atherogenesis. LPA is the most potent lipid component in oxLDL inducing SMC proliferation and migration. Furthermore, LPA induces SMCs to secrete inflammatory cytokines and chemokines, which may contribute to recruiting leukocytes into subendothelium and worsening vascular lesions. Hypertension is induced and neointimal formation promoted by LPA in the artery wall of rodents. LPA induction of TF expression in SMCs and platelet aggregation likely contribute to thrombus formation leading to atherothrombosis. Taken together, these accumulated *in vitro* and *in vivo* data strongly point to LPA's roles in promoting atherosclerosis and atherothrombosis. Efforts to manage LPA's effects on vascular disease are likely in the following aspects: LPA biosynthesis, metabolism, and signaling. LysoPLD/autotaxin has been reported as a major LPA synthesis enzyme in the blood and other enzymes are considered to produce LPA in local vascular lesions. LPA can also be metabolized into other molecules. These enzymes are targets for the future development of therapeutics. Furthermore, the identification of functions of individual LPA receptors, regulatory mechanisms of these receptor, and their downstream cellular effectors are also major targets for therapeutics. The selection of potent and specific inhibitors of LPA production and LPA degradation molecules, as well as LPA signaling effectors, are expected to prevent atherosclerosis and thrombosis.

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Executive summary

Lysophosphatidic acid synthesis & degradation in the vascular system

- Lysophospholipase D (LysoPLD) is considered the major lysophosphatidic acid (LPA)-generating enzyme.
- In blood circulation, LysoPLD mediation of LPA production is supported by evidence that heterozygous LysoPLD^{+/-} mice have about half the normal circulating levels of both LysoPLD and LPA and that transgenic overexpression of human LysoPLD in mice increases levels of plasma LysoPLD and LPA.
- In cells or vascular lesions, LPA can be converted from phosphatidic acid by phospholipase A₁/phospholipase A₂ activities.
- Degradation of plasma levels of LPA can be regulated by LPA degradation enzymes-lipid phosphate phosphatases.

Role of LPA in atherosclerosis & thrombosis

- LPA activates endothelial cells to secrete chemoattractant cytokines to attract monocytes and lymphocytes toward the endothelium of vascular walls.
- LPA stimulates the production of adhesion molecules on the surface of endothelial cells to tether and roll monocytes and lymphocytes to the endothelium. Adhesion and rolling of monocytes and lymphocytes to endothelium are initial steps of atherosclerosis.
- LPA induces smooth muscle cell (SMC) differentiation, proliferation, and migration, which are important features of atherogenesis.
- LPA is the most potent lipid component in oxLDL inducing SMC proliferation and migration.
- LPA induces SMCs to secrete inflammatory cytokines and chemokines, which may contribute to recruiting leukocytes into the subendothelium, worsening vascular lesions.
- Hypertension is induced and neointimal formation promoted by LPA in the artery wall of rodents. LPA has been demonstrated to promote atherosclerosis in an atherosclerotic mouse model.
- LPA induction of tissue factor expression in SMCs and platelet aggregation likely contribute to thrombus formation leading to atherothrombosis.

Conclusion

- These accumulated *in vitro* and *in vivo* data strongly point to LPA's roles in promoting atherosclerosis and atherothrombosis.

Figure 1. Lysophosphatidic acid chemical structure

Lysophosphatidic acid is a simple lipid molecule with a glycerol backbone, a phosphate residue and an ester-linked fatty acid with a variable length carbon chain that is either saturated or unsaturated. The fatty acid carbon chain can be linked at either the sn-1 position (as shown) or the sn-2 position of glycerol.

Figure 2. Effects of various lipids on smooth muscle cell migration and proliferation *in vitro* (A) LPA has the most prominent effect on SMC migration compared with other lipid components of oxLDL. LPA (different carbon chains), 18:1 LPA (1-oleoyl-2-hydroxysn-glycero-3-phosphate) and 16:0 LPA (1-palmitoyl-2-hydroxy-sn-glycero-3-phosphate) were used to stimulate mouse SMCs. SMC migration was measured as described previously [167]. (B) LPA has the most pronounced effect on SMC proliferation compared with other lipid components of oxLDL. LPE was used as a structure-related lipid control. SMC proliferation was measured as has been described previously [168]. HNE: Hydroxynonenal; LPA: Lysophosphatidic acid; LPC: Lysophosphatidylcholine; LPE: Lysophosphatidylethanolamine; oxLDL: Oxidized LDL; POVPC: 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine; SMC: Smooth muscle cell.

Figure 3. Lysophosphatidic acid's effects on vascular wall cells and blood cells, which have been found in atherosclerotic lesions and contribute to atherosclerotic lesion development and thrombosis

Anatomy of the arterial wall is illustrated (top left).

LPA: Lysophosphatidic acid; ROI: Reactive oxygen intermediate; ROS: Reactive oxygen species. Reproduced with permission from Deborah K Haines © 2010–2011 The University of Tennessee.

Figure 4. Lysophosphatidic acid induces neointimal formation in a noninjury rat model

The left internal carotid artery and the common carotid artery, distal to the bifurcation, were temporarily clamped. A transverse arteriotomy was performed in the left external carotid artery and a catheter was carefully inserted into the left external carotid without any injury to the endothelium of the common carotid artery. The common carotid artery was washed with the base medium (saline with 0.2% bovine serum albumin) and then filled with the base medium or 2 μ M LPA dissolved in the base medium through the catheter. After 30 min, the left external carotid artery was tied off, the clamps removed and blood circulation restored. After 20 days, the control rat (saline treated) and the LPA-treated rat were perfused intracardially with 4% paraformaldehyde. The carotid artery was excised and embedded in paraffin and the sections were stained with hematoxylin and eosin. LPA: Lysophosphatidic acid.