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Roles of Lysophosphatidic Acid in Cardiovascular Physiology and Disease

Susan S. Smyth^{1,2}, Hsin-Yuan Cheng², Sumitra Miriyala², Manikandan Panchatcharam², and Andrew J. Morris^{2,3}

¹ Department of Veterans Affairs Medical Center, Lexington, Kentucky 40511

² Division of Cardiovascular Medicine, The Gill Heart Institute

³ Department of Biochemistry, University of Kentucky, Lexington, KY 40536

Abstract

The bioactive lipid mediator lysophosphatidic acid (LPA) exerts a range of effects on the cardiovascular system that suggest a role in a variety of critical cardiovascular functions and clinically important cardiovascular diseases. LPA is an activator of platelets from a majority of human donors identifying a possible role as a regulator of acute thrombosis and platelet function in atherogenesis and vascular injury responses. Of particular interest in this context, LPA is an effective phenotypic modulator of vascular smooth muscle cells promoting the de-differentiation, proliferation and migration of these cells that is required for the development of intimal hyperplasia. Exogenous administration of LPA results in acute and systemic changes in blood pressure in different animal species, suggesting a role for LPA in both normal blood pressure regulation and hypertension. Advances in our understanding of the molecular machinery responsible for the synthesis, actions and inactivation of LPA now promises to provide the tools required to define the role of LPA in cardiovascular physiology and disease. In this review we discuss aspects of LPA signaling in the cardiovascular system focusing on recent advances and attempting to highlight presently unresolved issues and promising avenues for further investigation.

1. Introduction

Studies of vascular cells and cardiovascular physiology continue to provide important insights into both the basic biology and biomedical importance of signaling initiated by the bioactive lysophospholipid mediator lysophosphatidic acid (1-acyl 2-hydroxyl glycerol 3-phosphate, LPA). The very first experimental observations of biologic activity ascribed to LPA identified a role for this lipid as a regulator of smooth muscle cell contractility and the initial direct demonstrations of a physiological effect of exogenous administration of LPA came from studies in which this lipid was shown to modulate blood pressure in rodents and other mammalian species [93,94]. Similarly, studies of the agonist effects of LPA on human platelets were among the first indications that LPA might act through specific receptor systems which is an idea that has now culminated in the identification of six LPA-selective G-protein coupled

Corresponding authors Susan S. Smyth, M.D., Ph.D. and Andrew J. Morris, Ph.D., Division of Cardiovascular Medicine, The Gill Heart Institute, 900 S. Limestone Street, 326 CTW Building, Lexington, KY 40536-0200, 859-323-3749 (telephone), 859-281-4892 (fax), a.j.morris@uky.edu, susansmyth@uky.edu.

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receptors. Several reviews present this important work in its proper historical context and we refer the reader to these for further information [28,56,85,100]. At the same time, it would also be fair to say that the precise definition of the role of LPA in normal cardiovascular physiology and cardiovascular disease has lagged behind studies of the role of this lipid mediator in other settings most notably cancer and the nervous system. Because of the well established role of LPA as a pro-inflammatory stimulus for a variety of vascular cells, an activator of human platelets, a phenotypic modulator of vascular smooth muscle cells and a regulator of vascular tone, the potential for involvement of this lipid mediator in a variety of cardiovascular diseases is high. These could include roles in thrombosis, hypertension, and the initiation of intimal hyperplasia that accompanies vascular responses to injury and the progression of atherosclerosis. Testing these ideas will require a concerted approach in which the genetic and pharmacological tools developed for basic investigations of LPA signaling are combined with appropriate pre-clinical (animal) models and eventually translated to a clinical setting. In this review we discuss aspects of LPA signaling in the cardiovascular system focusing on recent advances and attempting to highlight presently unresolved issues and promising avenues for further investigation.

2. Regulation of Circulating Lysophosphatidic Acid Levels

The enzymes and pathways involved in the synthesis and inactivation of LPA have been reviewed in detail by others [4]. As discussed below, physiologically relevant levels of LPA can be detected in a variety of biological fluids including the blood. Accordingly, it is reasonable to presume that the steady state circulating LPA level is dictated by a balance between opposing pathways of LPA synthesis and LPA degradation. A variety of enzymes and pathways could be involved in the production of LPA by either *de novo* synthesis or by phospholipase-catalyzed hydrolysis of phospholipids. However, compelling evidence identifies a principal role for a secreted lysophospholipase D in the generation of bioactive LPA in the blood by hydrolysis of circulating or locally produced lysophospholipid substrates. Likewise, integral membrane lipid phosphatases that remove the phosphomonoester group from LPA to form monoacylglycerol appear to be key regulators of LPA metabolism [70]. Evidence for a phospholipase A-dependent pathway for generation LPA by hydrolysis of phosphatidic acid has also been presented but this interesting enzyme presently remains largely unexplored [5]. Another area of great potential importance is the distribution of LPA among protein and lipoprotein carriers in the plasma [34,86,86]. Finally, the recent finding that the lysophospholipase D responsible for the synthesis of LPA in the blood can bind to the surface of lymphocytes through interactions with integrin adhesion receptors [42] raises the possibility that localized production of LPA at the cell surface rather than bulk changes in systemic circulating LPA levels are important in cardiovascular regulation. Below we summarize our current understanding of the role of plasma enzymes, lipid substrates and the formed elements of the blood in the regulation of circulating LPA levels focusing on recent advances and unanswered questions.

3. Enzymology of LPA synthesis and inactivation in the blood

A series of studies initiated in the 1970s identified a circulating lysophospholipase D activity that could synthesize LPA by hydrolysis of lysophospholipid substrates in human and animal plasma. This enzyme has been identified as autotaxin which was originally described as a cancer cell motility factor [96,98]. Production of LPA clearly accounts for the ability of autotaxin to promote motility of cultured cells because these effects are augmented by exogenous provision of LPA and can be abrogated by genetic or pharmacological inactivation of LPA receptors on the target cells [37]. Homozygous inactivation of the autotaxin gene in mice results in early embryonic lethality but animals that are heterozygous for the wild type and null alleles have circulating LPA levels that are half those of wild type mice [92,101] while

transgenic over expression of autotaxin in mice increases circulating levels of autotaxin protein and LPA [26]. These results establish autotaxin as a central regulator of LPA levels in the blood. Circulating LPA levels closely parallel circulating levels of autotaxin which suggests that, *in vivo*, the enzyme is effectively saturated with substrate and therefore operating at or close to its V_{max} . This focuses particular attention on the source and nature of the autotaxin substrate which is likely to be lysophosphatidylcholine (lysoPC) derived in part as a byproduct of cholesterol esterification or perhaps more relevantly formed through the actions of A-type phospholipases acting on phospholipid substrates in platelets and other vascular cells. Circulating levels of lysoPC are very high in the blood (~100 μ M) which is close to or exceeds the K_m of autotaxin for this substrate measured *in vitro* [59] further supporting the idea that, although localized production of lysoPC substrate may promote LPA synthesis in some settings, bulk circulating levels of LPA would be largely unaffected by changes in circulating autotaxin substrates. An interesting conundrum is suggested by the finding that LPA can act as a mixed inhibitor of the lysophospholipase D activity of autotaxin *in vitro* suggesting that production of LPA by this enzyme may be “self-limiting” in some situations [98]. LysoPC can be formed as a byproduct of cholesterol esterification, but it is present in plasma from patients lacking the enzyme responsible and can therefore be formed by other pathways [4]. Of particular interest here is the possibility that activated platelets or other vascular cells can produce LPA through the actions of A-type phospholipases and the indirect role of platelets in LPA production in the plasma discussed below suggests a possible role in the provision of lysoPC substrate to autotaxin at sites of platelet activation.

Inactivation of LPA in the circulation likely involves activities of members of the lipid phosphate phosphatase (LPP) class of cell surface integral membrane proteins which are expressed in a variety of both circulating and blood vessel cells [11]. In particular LPP activity, protein and mRNA can be detected in a number of relevant cell types including platelets, leukocytes, vascular endothelial and vascular smooth muscle cells [82,87]. Inactivation of LPP2, one of the three mammalian LPP genes in mice, has been reported to produce no phenotype although circulating LPA levels have not been measured in these animals [111]. By contrast inactivation of the LPP3 gene results in early embryonic lethality but LPP3 heterozygotes are viable [25] and it will be interesting to see if circulating LPA levels are altered in these animals. Inactivation of the third LPP gene, LPP1, has not yet been reported, but transgenic mice that overexpress LPP1 have no change in circulating LPA levels [109].

4. Role of platelets in LPA production

As discussed in detail below, LPA levels in serum prepared from platelet-rich plasma have been reported to be ~5–10 –fold higher than in platelet-poor plasma which suggests that activated platelets play a direct or indirect role in LPA production during clotting. While several studies show that isolated platelets can produce LPA, which can be readily detected by radiolabeling strategies, the absolute quantities formed are too small to account for the mass changes in LPA observed during clotting. On the other hand, experimental induction of thrombocytopenia in rats using an anti platelet antibody decreased the production of LPA in serum by almost 50% [5]. Similarly, treatment of mice with a small molecule inhibitor of the platelet fibrinogen receptor resulted in a significant decrease in circulating LPA levels [9]. Interestingly, these experiments suggest that the primary role of platelets in LPA production in serum is indirect and may involve the localized generation of lysoPC as a substrate for production of LPA by the lysophospholipase D activity of autotaxin. A previous study suggested that microparticles released by activated platelets were a conduit for the production of extracellular LPA by platelets [27]. Taken together, these results suggest the interesting possibility that platelet derived microparticles may supply substrate for localized production of LPA at sites of platelet activation. Because autotaxin has recently been shown to bind to activated integrin receptors [42], the possibility that this enzyme is recruited to the surface of

platelets or platelet derived microparticles by the platelet fibrinogen receptor is clearly a very exciting idea.

5. Quantitation of plasma LPA levels

Considerable effort has been expended to develop and validate methods for quantitation of LPA levels in the blood because of substantial interest in the possibility that LPA may be a disease biomarker. Despite these efforts, reports of the absolute total mean concentrations of LPA in the circulation of healthy individuals vary by more than an order of magnitude (Table 1) suggesting that systematic differences in the efficiency of quantitation of LPA between the various assays used requires resolution. The ideal biomarker assay for LPA would be amenable to high throughput, could be conducted using readily available laboratory equipment, and would be able to measure different species of LPA with the required specificity and sensitivity to allow routine determinations in small samples. Several assays have been developed to measure LPA, including enzyme-based assays such as a radioenzymatic assay that measures the incorporation of fatty acyl coA of known specific radioactivity into LPA catalyzed by LPA acyl transferase [78] and a “cycling assay” in which LPA is converted into glycerol 3-phosphate by a specific lysophospholipase which is then quantitated by a colorimetric measurement of hydrogen peroxide formed by glycerol 3-phosphate oxidase [44]. These enzymatic assays produce measurements of plasma LPA levels in mice and humans of approximately 0.1 μM (Table 1) with an ~4-fold elevation observed in serum. Because the length, saturation and position of esterification of the acyl chains are a critical determinant of the biological actions of LPA these enzymatic assays, which cannot discriminate between these molecular species, would fail to detect interesting and potentially significant variations in the composition of the circulating LPA pool. Consequently, particular efforts have been made to develop mass-spectrometry based approaches for quantitation of defined LPA species. In this case, the most comprehensive measurements involve the use of reverse phase HPLC separation of LPA from other plasma lipids with electrospray ionization and tandem mass spectrometry using instruments with triple quadrupole mass analyzers (HPLC/ESI/MS/MS) [7,13,83,84,90,104,105,107]. Detection and quantitation of LPA relies on the production of a daughter ion corresponding to the glycerol phosphate head group with assignment of the acyl chain configuration made by reference to the parent ion. The HPLC step is critical to resolve LPA from interfering compounds that can produce the same parent-daughter ion transitions as LPA and optimization of these protocols has recently led to the report of methods with run times of ~10 minutes that are quite amenable to sample throughput rates that are compatible with the processing of clinical samples. Accordingly the field is now approaching a stage where definitive evaluation of circulating LPA levels as a disease biomarker should be possible and several other obvious areas for investigation such as the impact of anti-platelet drugs on circulating LPA levels should now be possible. The HPLC/ESI/MS/MS approach has several limitations that need to be considered when comparing data from different investigators. The efficiency of detection varies significantly between different LPA species which means that calibration is required for each of the species being measured and extrapolation of the efficiencies of detection of LPA species for which standards are available to “unknown” species is generally not appropriate. Moreover, the efficiency of detection of LPA in ESI/MS/MS is dramatically enhanced by the inclusion of acetate or formate anions in the mobile phase which can result in accumulation of this material in the ion source resulting in sample to sample variation in detection efficiencies which necessitates the frequent inclusion of calibration standards within a set of experimental unknowns. It’s also notable that measurement of the parent-daughter ion transition associated with formation of the glycerol phosphate product ion provides no information about the presence of 1-acyl or 2-acyl LPA species in the molecular ion. With these caveats in mind, based on reports from at least four separate laboratories it is notable that measurements of plasma LPA levels in mice and humans using HPLC/ESI/MS/MS consistently produce total LPA concentrations that are ~10-fold higher than those reported

by workers using the enzymatic assays although it is encouraging to note that, as with the enzymatic assays, these HPLC/ESI/MS/MS methods also detect LPA levels in serum that are elevated ~5-fold over matched plasma samples. Detection of at least 7 distinct LPA species has been reported in human plasma with a moderate consensus that 18:2, 20:4 and 16:1 LPAs are the most abundant. Another interesting but not yet fully explored observation is that measurable levels of 2-acyl lysoPC can be detected in the blood suggesting a role in the production of corresponding 2-acyl LPAs which may have a different spectrum of biological activities to those of the 1-acyl LPAs [58]. In this regard it is worth noting that spontaneous migration of the acyl chains of these lysophospholipids favors accumulation of the more stable 1-acyl species so concerns about sample preparation and of the inherent limitations of tandem mass spectrometry methods that will not distinguish 1-acyl from 2-acyl LPA species means that resolution of this interesting issue will require further methodological advances. Clearly then more work is needed to compare these types of LPA assays side by side and to harness their complementary strengths to explore how circulating LPA levels vary in health and disease.

6. Circulating LPA as a bioactive mediator of atherothrombotic disease

The work described above clearly establishes autotaxin as a principal regulator of plasma LPA levels and implicates activated platelets in the process of LPA production. The ability of autotaxin to bind to activated integrin receptors [42] suggests the possibility that the enzyme could be localized to the surface of activated platelets, which might concentrate the production of LPA at sites of vascular injury. LPA is also produced by mild oxidation of LDL and is found in the lipid-rich core of atherosclerotic plaques [86]. These observations position LPA as a potential biomediator of atherothrombotic vascular disease. Atherosclerosis is characterized by endothelial dysfunction, lipid accumulation in large and medium-sized arteries, vascular inflammation, and a fibroproliferative response of resident smooth muscle cells. Ultimately, erosion or rupture of the atherosclerotic plaque can trigger platelet-dependent thrombus formation, the proximate cause of most heart attacks and many strokes. In the following sections, we review the spectrum of effects that LPA has on vascular cell function that are consistent with its serving as a potential mediator of both inflammatory and thrombotic responses in the context of atherosclerosis.

7. LPA effects on platelet function

Some of the earliest observations of receptor-mediated actions of LPA came from studies in platelets [22,30–32,80,81]. LPA is a weak activator of human platelets and stimulates platelet shape change [31,74], fibronectin matrix assembly [62], and platelet-monocyte co-aggregate formation [38]. Fibronectin deposition and matrix formation on the surface of activated platelets in a growing thrombus may enhance thrombus formation and stability [15–17]. LPA also functions synergistically with other platelet agonists, such as epinephrine and adenosine diphosphate (ADP), to enhance platelet aggregation and adhesion [24,61]. Mildly-oxidized LDL stimulates platelet function at least in part in an LPA dependent manner [46,53,74,86] and platelet aggregation induced by components of atherosclerotic plaque lipid-rich cores can be inhibited by LPA receptor antagonists [77]. Thus, following plaque rupture or erosion, exposure of LPA in the lipid rich core or production of LPA at sites of platelet activation may serve a key role in triggering or potentiating platelet responses during acute thrombosis.

Human platelets contain mRNAs for five of the known G-protein coupled LPA receptors, LPA 1–5 [58,65], and in fact LPA4 and LPA5 are among the most abundant platelet G protein-coupled receptor transcripts [3], but the role of these defined receptor systems in mediating LPA responses in platelets has yet to be established. Highly unsaturated acyl-LPAs (22:6, 20:5, and 20:4) and 16:0 LPA are more potent activators of human platelet aggregation than LPAs having a C18 fatty acid group, and alkyl-LPAs are the most potent platelet activators [35,88,

97]. This unusual structure-activity relationship is not shared by LPA1–4, suggesting that these receptors are unlikely to be primary mediators of the major stimulatory effects of LPA on human platelets. Thus, either the stimulatory responses are mediated by LPA5 or by an as yet unidentified LPA receptor on platelets. LPA induces human platelet shape change by activation of the GTPase Rho and its downstream target Rho kinase, which in turn stimulates myosin light chain kinase and moesin phosphorylation to regulate actin organization and shape change [73]. Other platelet agonists act through receptors coupled to G_{12/13} to activate Rho/Rho kinase and trigger shape change [45], and LPA receptor(s) coupled to G_{12/13} may elicit the same pathway. LPA also stimulates the tyrosine kinases Src and Syk in platelets [53], which can result in phosphorylation of actin-binding proteins such as gelsolin [55]. Furthermore, the addition of LPA to permeabilized platelets can dissociate actin from actin binding protein complexes, suggesting that it may function as an intracellular regulator of the actin cytoskeleton [55]. Reports differ with regard to the extent to which LPA elevates intracellular Ca²⁺ in platelets [53,66,73], but Ca²⁺ influx is not required for activation of either Rho or Src by LPA. Higher concentrations of LPA can elicit platelet granule secretion in a fibrinogen-dependent manner [66], suggesting that engagement of the integrin α IIB β 3 and possibly also platelet-platelet interactions are required for secretion. ADP receptor antagonists block LPA-induced platelet aggregation in whole blood, indicating that released ADP may potentiate platelet LPA responses [38]. The concentrations of LPA required to elicit platelet aggregation in whole blood are higher than those necessary to stimulate activation of isolated platelets. LPA binding proteins in plasma, such as albumin or gelsolin [63], may contribute to this effect by modifying platelet LPA responses. Importantly, as discussed above, LPA production may normally be localized at the platelet surface or on microparticles in close proximity to platelet cell-surface receptors. Platelets contain LPPs and chemical inhibitors of LPP activity potentiate the effects of LPA[89]. LPA has also been proposed to serve as an endogenous activator of the nuclear peroxisome proliferator-activated receptor gamma (PPAR γ) [54]. Although platelets are anucleate, they contain and release PPAR γ upon activation [1,71,72] and PPAR γ agonists block agonist-induced release of CD40L and thromboxane B2 from platelets. Thus, in addition to the stimulatory pathways describe above, platelets may also possess machinery that links LPA to inhibitory responses. Indeed, platelet responses to LPA are highly variable and donor-dependent [24,38], and individuals whose platelets selectively fail to respond to LPA have been identified [65,97]. The presence of LPA-mediated inhibitory signals is one possible mechanism that could explain these variable platelet responses to LPA. Finally, LPA does not appear to activate or induce aggregation of rodent platelets [81], suggesting that either rodent platelets lack key LPA stimulatory systems or LPA-inhibitory mechanisms may predominant in rodent platelets. The fundamental difference in LPA responses between rodent and human platelets warrants consideration when assessing atherothrombotic responses in murine models.

8. LPA effects on immune cell function

While the role of SIP in regulation of immune cell function by modulation of leukocyte trafficking is well-established, the immunomodulatory effects of LPA are less clear. LPA receptors are expressed on lymphocytes[33,103], dendritic cells [69], and in lymphoid organs. LPA promotes chemotaxis of human dendritic cells [69] and activated T-cells [103] and, in the case of immature murine dendritic cells, the effects are mediated by LPA3 [12]. LPA promotes integrin-dependent adhesion of splenic B cells [75]. Recent data indicates that autotaxin is highly expressed and secreted by high endothelial venules of lymphoid organs, and secreted autotaxin binds to lymphocytes in an integrin-dependent which may generate LPA at the cell surface and in turn promote the entry of lymphocytes into lymphoid organs [42]. LPA also activates cells involved in innate immunity including neutrophils [14,41], eosinophils [40] and mononuclear phagocytes [48]. Of particular interest from the standpoint of understanding atherothrombotic effects in the vasculature, LPA stimulates neutral lipid accumulation in monocyte-derived cells, and prevents reverse transmigration of monocytes,

suggesting that LPA in atherosclerotic plaque may prevent monocyte egress from the vessel wall and promote plaque progression [52].

9. LPA effects on endothelial cells

Activation of the endothelium plays a prominent role in early atherosclerotic development. As is the case with immune cell regulation, in comparison with our knowledge of SIP effects on endothelial cell function, much less is known about the actions of LPA on the endothelium. LPA promotes endothelial cell migration through mechanisms that involve regulation of the actin cytoskeleton and the extracellular matrix [6,68]. LPA has been variably associated with increasing or decreasing endothelial barrier function but the differences between these sets of observations may arise from differences between the types of endothelial cells, the source and presentation of LPA, and the methodologies used to probe barrier function used in these studies [67]. For example, several investigators report that LPA stabilizes endothelial barrier function [23], decreases endothelial permeability [2], and increases endothelial resistance [57], whereas others have found that LPA promotes a loss of vascular integrity and decreases transendothelial resistance by preventing tight junction formation [60,102]. In lung injury models, vascular leak is mediated by the LPA1 receptor [91]. In cell culture systems, LPA triggers an inflammatory response in endothelial cells that includes upregulation of expression of leukocyte chemoattractants [50] and adhesion receptors [76] in an LPA1 and LPA3 –dependent manner [50,51]. These responses can regulate endothelial-leukocyte interactions by promoting monocyte binding to endothelial cells [76]. Additionally, the secretome of LPA-treated endothelial cells has been profiled, and a major secreted glycoprotein that is produced in response to LPA is pentraxin-3, an acute phase reactant that promotes monocytic-like cell chemotaxis [36]. LPA also activates eNOS in endothelial cells [47] and may thereby regulate endothelial tone. Given the range of effects elicited by LPA *in vitro*, it is both conceivable and attractive to speculate that increases in local or systemic circulating LPA levels could regulate on endothelial cell function *in vivo* in a manner that impacts atherogenesis.

10. LPA effects on smooth muscle cells

Phenotypic modulation of vascular smooth muscle cells (SMCs) occurs in response to vascular injury and is a critical component in the development of atherosclerotic and restenotic lesions [43,64]. Phenotypic modulation of vascular cells involves their conversion from a normally quiescent, differentiated, “contractile” state to a dedifferentiated state in which the cells proliferate, migrate and synthesize matrix proteins. As described above, LPA may be produced at sites where activated platelets accumulate, such as along injured vessels, and LPA can be generated during oxidative modification of LDL and is found in abundance in the lipid-rich core of atherosclerotic plaque. Thus, LPA is present or can be formed in the settings associated with phenotypic modulation of SMCs. Isolated vascular SMCs from human and rodent species can be stimulated to dedifferentiate, proliferate, and migrate by serum, and LPA has been proposed as one of the factors present in serum that may promote phenotypic modulation of vascular SMCs [39,85,106]. Indeed, isolated vascular SMCs respond to LPA by proliferating [29,79,95] and migrating [8,20]. Although the specific LPA receptors and their signaling systems involved in SMC responses are not known, prominent pathways that are activated by LPA include Rho GTPases and extracellular-signal regulated kinase (ERK). LPA also regulates expression of early growth response-factor (Egr-1), a transcription factor, that activates expression of proinflammatory cytokines, adhesion molecules, growth factors, and coagulation factors [18]. LPA can promote tissue factor expression by SMCs [19], which may be a key component of the vessel wall involved in triggering thrombus formation [21].

Exogenous administration of LPA to animals elicits responses consistent with it serving as an endogenous mediator of vascular cell function. For example, intravenous injection of LPA

elevates arterial blood pressure in rats [94] and local application causes cerebral vasoconstriction in pigs [93]. Moreover, local infusion of LPA in the rat common carotid artery induces vascular remodeling by stimulating neointimal formation [108]. A similar response is observed in mice and is proposed to be mediated by PPAR γ [110]. The finding that LPA is capable of eliciting the development of intimal hyperplasia, which involves phenotypic modulation of SMCs, suggests that it may normally regulate SMCs, and we have observed differences in the development of neointimal hyperplasia in mice lacking the LPA1 receptor and in mice lacking both LPA1 and LPA2, implicating endogenous LPA in phenotypic modulation of SMC after vascular injury (Panchatcharam M, Morris AJ, and Smyth SS, unpublished data).

11. Evidence for Cardiovascular regulation by LPA in humans and animal models

The findings summarized above establish that LPA can regulate growth, differentiation, survival, motility, and contractile activity of a variety of vascular cells *in vitro*. Based on the results of studies with isolated or cultured cells, LPA would appear to be poised to serve as a key bioactive mediator of inflammatory and thrombotic responses and to be a pathophysiologic modulator of vascular cell function. Observations in human disease and animal models of human disease support this contention. For example, the toxic factor in the venom of the brown recluse (*Loxosceles*) spider is a sphingomyelinase D with phospholipase D activity that generates LPA [49,99]. Brown recluse spider bites produce local and systemic responses that involve inflammation, thrombosis, vascular leakage and intravascular hemolysis, and in severe cases renal failure, suggesting that overproduction of LPA can have dire vascular consequences. On the flip side, mice deficient in the lysophospholipase D autotaxin have impaired blood vessel formation and die embryonically, likely from a defect in autotaxin- and LPA-mediated vessel maturation [92,101]. Mice lacking LPA receptors have wild type or mild phenotypes, suggesting considerable functional redundancy between these receptors or the presence of yet-unidentified-receptors. Additional roles for the LPA receptors in the cardiovascular system may be revealed as mice lacking individual LPA receptors are carefully assessed in disease models and of course here the interplay between cardiovascular cells and cell types involved in other disease processes is particularly interesting. For example, studies using a mouse model implicate LPA as a mediator of platelet interactions with circulating breast cancer cells that ultimately promote promoting breast cancer metastasis to bone [9,10]

12. Unresolved questions

This is an exciting time in the field where a series of technical advances in methods for measurement of LPA and the identification of genes encoding key enzymes responsible for LPA metabolism make it possible to address a number of key issues relating to the regulation of LPA in the cardiovascular system. The first and perhaps most obvious of these is to examine the metabolic heterogeneity of the plasma LPA pool. The half life of circulating LPA is presently not known and it seems likely that because the total level of LPA in the plasma significantly exceeds the concentrations required for activation of LPA receptors on cultured cells a significant fraction of this circulating LPA is sequestered and or inactive, perhaps as a result of binding to plasma proteins. As noted above, the close relationship between circulating LPA levels and circulating autotaxin levels suggests that control of substrate supply to this enzyme is an important regulator of bulk levels of LPA in the blood. The recently described binding of autotaxin to activated integrin receptors clearly focuses attention on the possible role of this enzyme in localized production of LPA[42] In particular recruitment of autotaxin to integrin receptors on activated platelets could result in localized production of LPA at sites of vascular injury or plaque rupture. Furthermore, although circulating autotaxin levels are low in comparison to the primary ligands for these receptors this would suggest that the class of

antiplatelet drugs which target these integrin receptors might interfere with the recruitment of autotaxin to platelets and could thereby inhibit platelet-dependent production of LPA in the blood. Finally, a causative role for LPA in the development of complications of atherosclerosis remains to be established. In addition to observations in animal models of human disease, proof for a causative role for LPA in cardiovascular disease will require data establishing that levels of LPA and/or autotaxin activity change during disease and that manipulations of LPA levels and/or autotaxin activity reduce or prevent atherothrombosis. Finally the development of experimental therapeutics targetting LPA receptors and enzymes responsible for the synthesis and inactivation of LPA continues to be a fruitful area of research and the recent description of small molecule inhibitors of autotaxin may provide both a valuable tool to investigate LPA function *in vivo* and perhaps an entry point to the development of ways to intervene in LPA signaling in the cardiovascular system with potential benefits for cardiovascular disease.

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Table 1

Quantitation of LPA in plasma and serum

Refer to references cited for experimental details. Values are from blood obtained from human sources unless otherwise noted. Plasma was obtained from blood anticoagulated with either citrate or EDTA

Method	Plasma LPA Mean±SE (μM)	Serum LPA Mean/Range (μM)	Molecular Species (Plasma)	Notes	Reference
Radioenzymatic assay	0.083±0.08 (Human) 0.17 ±0.05 (Mouse)	0.28–0.71 (Fetal, Bovine) 1.31–2.56 (Donor, Bovine)	ND	Assay involves acylation of LPA catalysed by bacterial LPA acyltransferase using fatty acyl CoA of known specific radioactivity	Saulnier-Blache et al <i>J. Lipid. Res.</i> (2000) 41, 1947–1951.
Coupled Enzymatic Assay	0.08±0.02(♂) 0.09±0.02(♀)	0.41±0.14(♂) 0.41±0.12(♀)	ND	Assay involves lysophospholipase treatment and determination of glycerol 3-phosphate by an oxidase-coupled cycling reaction	Kishimoto et al (2003) <i>Clin. Chem. Acta</i> 33 59–67
TLC-Gas Chromatography	0.6±0.19(♀, Healthy) 14.9±3.22 (♀ Gynecologic Cancers)	ND	16:0, 18:0>18:1, 18:2, 20:4	TLC purification, quantitation of fatty acid methyl esters	Xu et al <i>JAMA</i> 1998 280 719–723 Shen et al. <i>Gyn. Onc.</i> 83 25–30
TLC/ESI/MS/MS	0.9±0.43(♀, Healthy) 2.57 0.94 (♀ Ovarian I) 2.15 0.71 (♀ Ovarian II) 2.93 1.77 (♀ Ovarian III) 1.97 0.27 (♀ Ovarian IV)	ND	20:4>18:2, 18:1>18:0, 16:0>22:6	TLC purification, direct infusion comparison of healthy controls with stage I-IV ovarian cancer patients	Supphen et al (2004) <i>Cancer Epidemiol. Biomarkers Prev.</i> 13 1185–1191
HPLC/ESI/MS	0.61±0.14(♂ Fresh) 0.91±0.23 (♂, 24hrs, 25°C) 0.74±0.17(♀, Fresh) 0.99±0.38 (♀, 24hrs, 25°C)	0.85±0.22(♂ Fresh) 1.57 ±0.56(♂, 24hrs, 25°C) 4.78 ±0.89(♀, Fresh) 5.57±0.73(♀, 24hrs, 25°C)	18:2>18:1>18:0, 16:0, 20:4	HPLC separation with LPA species identified by retention time and molecular m/z	Baker et al (2001) <i>Anal. Biochem.</i> 292, 287–295
ESI/MS/MS	4.3–5.0(♀, Healthy) 7.9–8.7(♀, Ovarian Cancer)	ND	16:0>18:2>18:0 0>18:1, 20:4	Direct infusion of total lipid extracts	Yoon et. al. J. Chromatograph. B. (2003) 85–92.
HPLC/ESI/MS/MS	ND	2.9 (♀, Healthy) 8.4 (♀, Ovarian Cancer)	ND	No plasma measurements reported	Melah et. al. J. Chromatograph. B. (2007) 287–291.
HPLC/ESI/MS/MS	1.244±0.262 (♀, Healthy) 1.307 ±0.355 (♀, Benign) 1.224 ±0.386 (♀, Cancer)	ND	16:0>18:2>20:4>18:1>18:0	Compared plasma LPA concentrations in healthy women to women with either benign or malignant breast tumors	Murph et. al. (2007) <i>Methods Enzymol</i> 433: 1–25
HPLC/ESI/MS/MS	No pre-purification 1.0–1.689 (♀, Healthy) 0.951–2.026 (♀, Cancer) TLC pre-purification 0.542–1.416 (♀, Healthy) 0.971–2.407 (♀, Cancer)	ND	18:2>16:0, 20:4>18:1, 22:6, 18:0	Compared plasma LPA concentrations in healthy women to those with ovarian cancer. Also examined effect of pre-purification of LPA by thin layer chromatography	Shan et. al. (2008) <i>J. Chromatograph. B</i> 864: 22–28.