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REVIEW ARTICLE

EVALUATION OF THE SIGNALLING MECHANISMS OF LYSOPHOSPHATIDIC ACID AND ITS ROLE IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

Lysophosphatidic acid (LPA), which is proposed to play an important role in normal physiological situations such as wound healing, maintenance of vascular tone, vascular integrity and reproduction, may also be implicated in the etiology of some diseases such as atherosclerosis, cancer, stroke and dementia. There is a causative relationship between stroke and dementia also leading to the classical Alzheimer's disease. Abnormal findings, including silent brain infarction, are frequently observed by magnetic resonance imaging in patients with nonvalvular atrial fibrillation. The importance of LPA in stroke and vascular cognitive impairment has been reported. This review article is focussed on the signalling mechanisms of lysophosphatidic acid as well as its role in central nervous system.

Key Words: Stroke, dementia, lysophosphatidic acid, metabolism, nervous system

INTRODUCTION:

In the recent years, a interaction has been found between vascular disease and cognitive impairment in elderly patients with mental illness (1). Therefore, it is important to identify modifiable vascular risk factors, which could be used in preventing or delaying the onset of dementia. New evidence has emerged that proper control of vascular disorders and maintenance of active lifestyle may help prevent or delay the onset and progression of dementia (2). The pathogenesis of ischemic stroke includes embolism, atherosclerosis, thrombus formation, infarction-related necrosis, and reperfusion injury after recanalization (3). In addition to conventional cardiovascular disease risk factors, a variety of inflammatory cytokines or biomarkers are now considered to contribute to the development of ischemic stroke. Indeed, it has been proven that atherosclerotic lesions contain monocyte-derived macrophages and T lymphocytes and leukocytosis is associated with plaque thickness in the carotid artery. Moreover, leukocytes may jeopardize the integrity of the endothelium which is important for the function of the blood brain barrier (4). There is now accumulating evidence that leukocyte-mediated vascular inflammation is implicated in the pathogenesis of ischemic stroke.

Several studies have found that lysophosphatidic acid (LPA), as a multifunctional "phospholipid messenger", is produced mainly by activated platelets and may be an ideal molecular marker reflecting platelet activation state *in vivo*. Platelets contribute to the production of LPA (5), which has been proposed to be a primary lipid in atherosclerotic plaque that is responsible for platelet activation (6). LPA is also abundant in human atherosclerotic plaques, where it is thought to be derived, at least in part, from mild oxidation of low density lipoproteins. Platelet depletion or treatment with anti-platelet agents reduces circulating LPA levels in animals (7). Hence, anti-thrombotic therapy targeting LPA production or receptor-mediated signaling may be a novel strategy to prevent thrombus formation. Silent brain infarction (SBI), which increases vascular cognitive impairment and stroke risk, has a high incidence in elderly people (8,9).

Since several clinical and epidemiological studies have reported during the last 30 years that show that even mild hyperhomocysteinaemia is associated with vascular disease (10), A investigation in elderly patients with mental disease with regard to plasma homocysteine (tHcy) and the presence of vascular disease was done. Briefly these findings showed that patients with mental illness and any forms of vascular disease had significantly

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higher plasma tHcy concentrations than patients without vascular disease, and that elevated plasma tHcy in mental illness was mainly associated with the presence of vascular disease and not related to the specific psychogeriatric diagnosis.

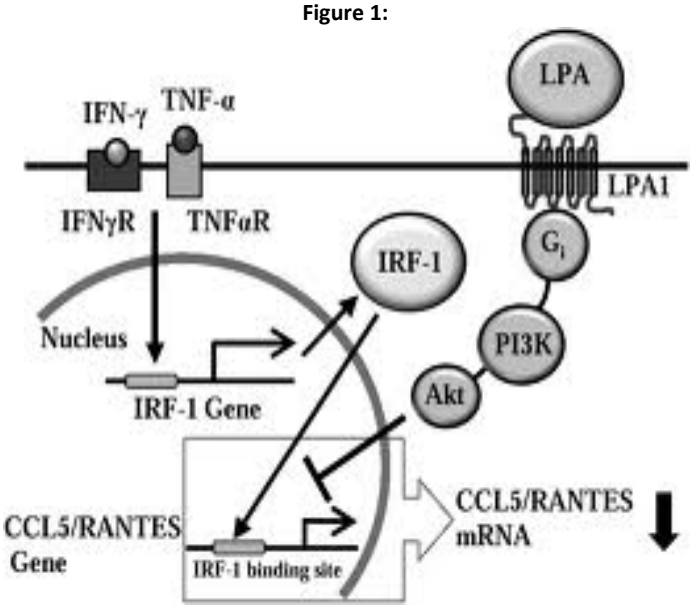
Finally, certain diseases may influence activated LPA release, including inflammatory processes, hypercholesterolemia or diabetes mellitus. This review article is focused on the signalling mechanisms of LPA and its importance in the central nervous system.

Synthesis of LPA and LPA receptors:

Although the exact mechanism of LPA metabolism within most types of cells is still unclear, two general pathways of LPA production have been demonstrated. In the first pathway phosphatidic acid (PA) is produced from phospholipids by phospholipase D (PLD), also called autotaxin (ATX) or from diacylglycerol by diacylglycerol kinase (11). In both these pathways there is deacylation of PA to LPA by phospholipase (PLA)-type enzymes. In the second pathway, are first converted to lysophospholipids by the action of secretory ($sPLA_2$), PS-PLA_1, and lecithin-cholesterol acyltransferase (LCAT), and then the LPL is converted to LPA by ATX . The first pathway is mainly involved in cellular LPA production, while the second pathway is involved in LPA production in extracellular body fluids, especially in serum and plasma. These various methods of LPA synthesis reflect multiple levels of regulation—or deregulation in the organism being at different physiological or pathological status—cancers, pregnancy, hypertension, stroke and dementia (12,13). Moreover, LPA-dependent different signaling pathways have therapeutic repercussions since pharmaceutical drugs targeting certain enzymes would differ from those targeting other LPA biosynthetic pathways.

In mammals, LPA exerts its action via at least six high affinity, transmembrane G-protein-coupled receptor (GPCR) types, LPAR1–LPAR6, and possibly through a nuclear receptor PPARγ These LPARs are expressed in various organs and cells (14,15). Refer Fig.1.For example, LPAR1 is highly expressed in the nervous system, LPAR2 in immune organs such as the thymus and spleen, and LPAR3 in reproductive organs such as the ovary and uterus. On the other hand, LPAR4, LPAR5, and LPAR6 are expressed widely but at relatively low levels. In that aspect LPAR4 expression in the ovary can be found (16). LPAR5 expression in the small intestine, spleen, dorsal root ganglion, and embryonic stem cells (17,18). However, there is also much evidence of an aberrant expression of LPA receptors in certain diseases, meaning that is pertaining especially to different types of cancer (19) as depicted in Figure 3.

Figure 1:



Estimation of LPA by LC-MS:

Mass Spectrometry Panels of LPA species including C16:0, C18:0, C18:1, and C20:4 are analyzed by using an LC-MS/MS method modified from the published method by Chen *et al* in 2008, (20). In brief, 50µl of sample was extracted with 350 µl of acetonitrile/methanol/acetic acid (50:50:1) containing C17:0 LPA at 5 ng/ml as internal standard. Then, 300 µl of supernatant was removed and 700 µl of methanol/water/TEA (66:34:0.1) is added before analysis.

Quantitation experiments can be performed by two-dimensional LC-MS/MS by using an HP 1200 LC system (Agilent Technologies, Santa Clara, CA) composed of a quaternary pump, a CTC Analytics HTS PAL autosampler (LEAP Technologies, Carrboro, NC), and a switching valve, plumbed in-line with a HP 1100 high-performance liquid chromatography pump that was interfaced to an API 4000 Qtrap mass spectrometer (MDS-Sciex, Toronto, Canada) operated in the negative ion electrospray and multiple-reaction monitoring modes. Typically, 300 µl of sample was injected onto a Betasil C18 column (Thermo Fisher Scientific, Waltham, MA) with 1 ml/min of 25 mM ammonium acetate/methanol (50:50) mixture. After 3 min, the valve is switched on, and the captured analytes are eluted onto a 3.0 X 50-mm, 3.5-µ m (particle size) XBridge C8 analytical column (Waters, Milford, MA) with a 3-min gradient at a flow rate of 0.8 ml/min. Gradient mobile phases used are methanol/methylene chloride/water (55:5:40) with 0.1% TEA and methanol/methylene chloride (50:50) with 0.1% TEA. The data can be analyzed using a soft ware programme.

LPA in the nervous system:

The LPA_1 receptor was originally characterized from neuronal progenitor cells in the ventricular zone of the

developing brain (21). LPA is important in myelination and Schwann cell survival (22,23). Perhaps the most profound morphological effect of LPA is on the development of cortical folds that lead to the development of gyrus in the cerebral cortex Refer Fig.2. There are several articles depicting the role of LPA in the brain, and these have a depth coverage of this exciting aspect of LPA developmental neurobiology (24).

Autotaxin (ATX) ; and the bioactive compound is LPA, the product of ATX-mediated LPC hydrolysis. LPA initiates neuropathic pain and regulates the production of pain-related molecules through the LPA_1 receptor (25,26). It has recently been proposed by Ueda *et al* that a feed-forward loop that involves LPA_3 receptor-mediated production of lysophosphatidylcholine, which supplies the substrate to ATX, rich in the cerebrospinal fluid. These investigators showed that LPA produced by ATX in turn could induce LPA_1-mediated neuropathic pain (27).

LPA_1 up-regulates the voltage-gated Ca^{2+} channel in the dorsal root ganglion cell, through a Rho-Rho kinase-coupled mechanism and produces demyelination of the fibres originating from the ganglion cells and leads to the reorganization of Aβ-fibres (28). In addition to the LPA_1 receptor, LPA_5 has also been implicated in neuropathic pain, although the mechanism responsible remains to be definitively established (29,30). New Antagonists are currently being developed targeting $LPA_1$and LPA_5 receptors might provide ways to treat this currently difficult-to-manage neurological condition. Perhaps more than a curiosity is the fact that B lymphocytes from patients with bipolar disorders are hyper-responsive to LPA (31); and in patients with schizophrenia, LPA_1 is among 18 genes that show close association with the disease (32).

LPA metabolism:

LPA has been found to be present in all eukaryotic tissues examined. The formation of an LPA species depends on its precursor phospholipid, which can vary by acyl chain length and degree of saturation. The term LPA most often refers to 18:1 oleoyl-LPA (1-acyl-2-hydroxy-sn-glycero-3-phosphate), as it is the most commonly used laboratory chemical entity. However, there is a growing range of recognized chemical forms of LPA in various biological systems that have been observed in concentrations spanning low nanomolar to micromolar levels. LPA concentrations in blood can range from 0.1 µM in plasma and up to 10 µM in serum, which is well over the apparent nanomolar Kd of LPA1-6. The 18:2, 20:4, 16:1, 16:0, and 18:1 LPA forms are particularly abundant in plasma (33). LPA degradation is mediated by several classes of enzymes, including three types of lipid phosphate phosphatases (LPPs), LPA-acyltransferase (LPAAT), and various phospholipases (e.g., PLA1 or PLA2). LPA may be converted back to phosphatidic acid by LPAAT, hydrolyzed by LPP (1-3), or converted by phospholipases into glycerol-3-phosphate.

Anti-LPA antibody as a strategy for therapy:

The Neutralization of extracellular LPA signaling could be the first therapeutic agent to mitigate both primary and secondary phases of neurotrauma, with resulting potential beneficial outcomes in rehabilitation and functional recovery for the patients (34). A variety of therapeutic interventions in the LPA signaling pathway can be envisioned and have been discussed previously. The development of small molecule inhibitors targeting either the LPA receptors or key enzymes in the biosynthetic machinery responsible for LPA production. Regarding LPA receptor antagonists, there is the problem that there are six G protein-coupled receptors (GPCRs) for LPA plus two purinergic receptors and possibly one ion channel (transient receptor potential cation channel subfamily V member 1 (TRPV1). This receptor redundancy may require the development of a pan-LPA receptor antagonist, as there are many LPA receptors expressed in the human brain after neurotrauma. This does pose a challange for medicinal chemistry. Alternately, one can target the upstream biosynthetic machinery responsible for LPA upregulation in the CNS. Although autotaxin is a major source of LPA in the blood and some tissue, it may not be the source of dysregulated LPA after neurotrauma and future research work will be required to determine the role of LPA production including phospholipase $(PLA)_1$, PLA_2, alpha glycerol kinase (AGK) and other monoacylglycerol (MAG)-kinases as well as glycerol-3-phosphate acyltransferase (GPAT) (35). Lysophosphatidyl choline (LPC), the substrate for LPA production by ATX, in a lower concentration in human CSF even after TBI (50 nM) and is consistent with previous reports of low LPC in normal CSF, and it can be argued that ATX may not be the key enzyme responsible for LPA production.

The humanized antibody could serve as a potential therapeutic agent for neurotrauma by limiting the initial injury and, at the same time, reducing dangerous inflammatory processes, possibly closing down the permeabilized BBB while stimulating regenerative processes and also by blocking the LPA receptor (Refer Fig.3). Thus, neutralizing extracellular LPA with the humanized mAbs could be the first therapeutic agent that mitigates both early and late phases of neurotrauma with resulting potential beneficial outcomes in rehabilitation and functional recovery for patients.

LPA and dementia:

The important enzyme that governs the synthesis of LPA is lysophospholipase D (lysoPLD), which is identical to

autotaxin, a tumor cell motility-stimulating factor (36). In this regard, it is important to note that a recent study revealed that autotaxin expression is enhanced in frontal cortex of AD patients, suggesting that autotaxin and its product LPA may be involved in AD pathology (Refer Fig.2) (37). Interestingly, in an effort to study the pathogenetic effect of oxLDL, new data revealed that LPA, the major bioactive component of oxLDL, can enhance Aβ production. This result prompted researchers to further determine the mechanism by which LPA upregulates Aβ production. As the Aβ peptide is produced from its precursor APP, it was first examined whether LPA treatment has any effect on APP expression. The resulting data revealed that LPA treatment has no effect on APP expression under experimental conditions. There are two enzymes playing key roles in APP processing and Aβ production, the β-secretase, which produces the N-terminal of Aβ, and γ-secretase, which produces the C-terminal of Aβ. Recent experimental data strongly supports the notion that different isoforms of PKC may play different roles in regulating APP processing, and specifically that, different from most other PKC members, such as PKCα, PKCβ, and PKCε, which are implicated in regulating α-secretase-mediated APP processing, PKCδ, a member of the novel PKC subfamily, is involved in LPA-induced upregulation of β-secretase expression and Aβ production (38).

CONCLUSION:

In the past 25 years we have seen the cloning and identification of a growing family of LPA receptors and signaling pathways, coupled with a rapidly burgeoning field aimed at understanding the physiological and pathophysiological importance of these lipids. It is now an exception, rather than the rule, to find an organ system that has not been impacted by LPA and LPA signaling. There is also a ever-growing understanding and appreciation that numerous human disease mechanisms are explicitly linked to altered LPA signaling. Perhaps most exciting is the development of novel pharmacological modulators that serve as both research tools and potential medicinal therapies aimed at reducing human suffering.

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Figure 2:

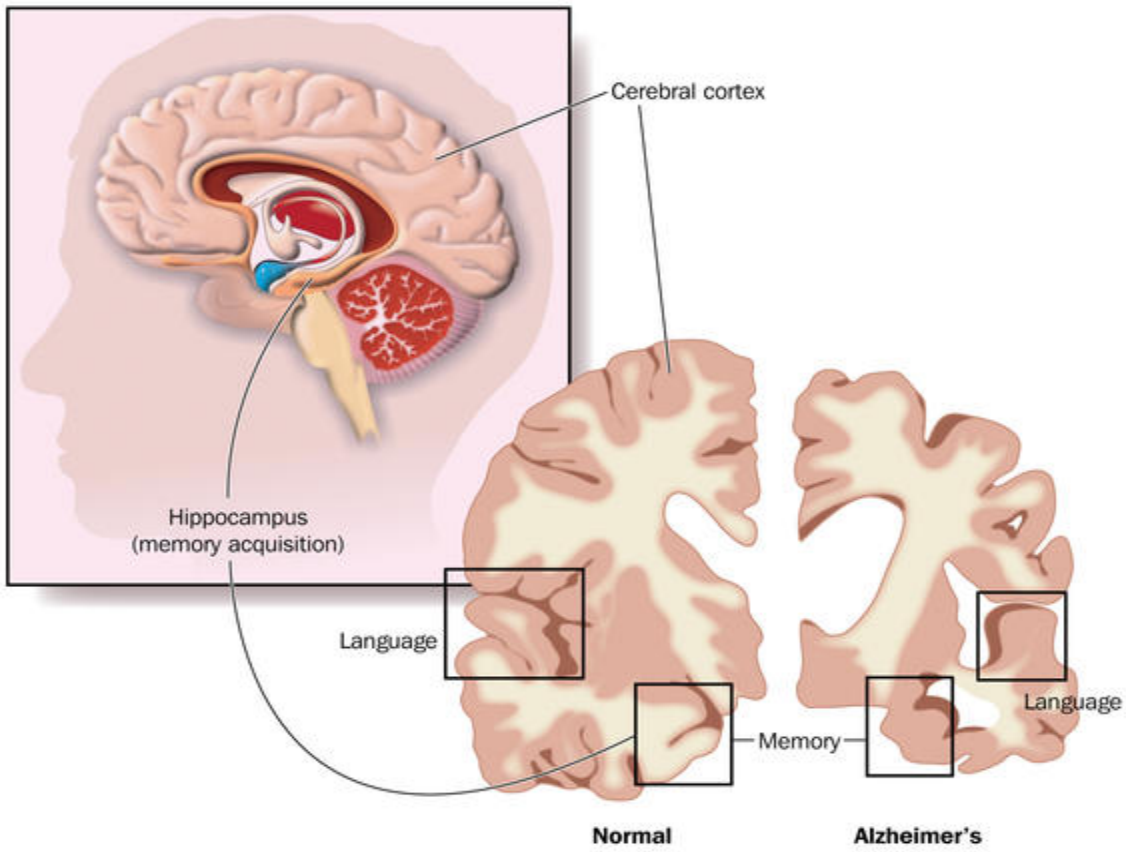
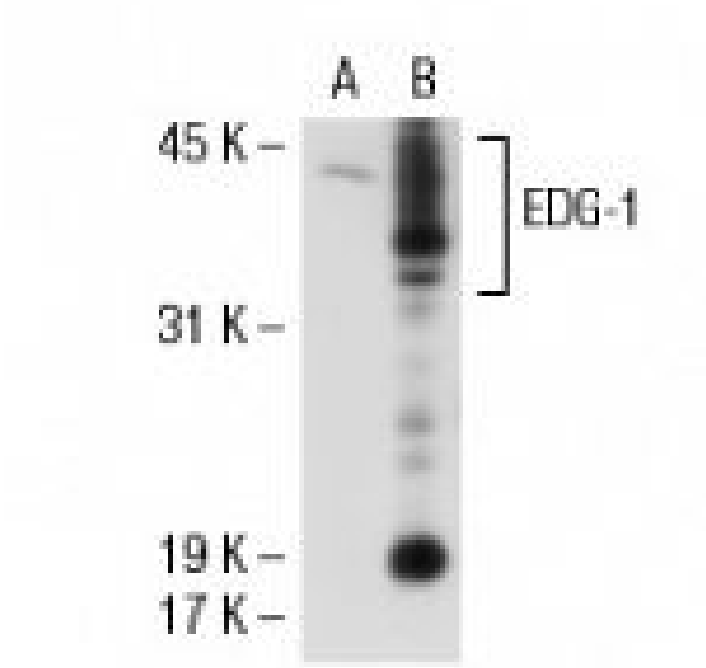


Figure 3: Western immunoblotting of LPA receptor using a EDG-1 H-60 antibody (Courtesy, Santa Cruz Biotechnology product catalog, USA)



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