Lysophosphatidic acid levels in cerebrospinal fluid and plasma samples in patients with major depressive disorder

Leo Gotoh [a], Misa Yamada [a], Kotaro Hattori [b,c], Daimei Sasayama [b,d], Takamasa Noda [e], Sumiko Yoshida [e,f], Hiroshi Kunugi [b], Mitsuhiko Yamada [a,*]

[a] Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo, 187-8553, Japan
[b] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, 187-8502, Japan
[c] Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, 187-8551, Japan
[d] Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, Japan
[e] National Center of Neurology and Psychiatry Hospital, Tokyo, 187-8551, Japan
[f] Medical Genome Center, National Center of Neurology and Psychiatry, Tokyo, 187-8551, Japan

ABSTRACT

Major depressive disorder (MDD) is the most common psychiatric disorders. However, a biochemical marker has yet to be established for clinical purposes. It is proposed that lysophosphatidic acid (LPA, 1-acyl-2-sn-glycerol-3-phosphoate) plays some important roles in emotional regulation of experimental animals. Therefore, in this study, we measured LPA levels using enzyme-linked immunosorbent assays of cerebrospinal fluid (CSF) and plasma samples from patients with MDD. The participants were 52 patients and 49 normal healthy controls for CSF study, and 47 patients and 44 controls for plasma study. We used the Japanese version of the GRID Hamilton Depression Rating Scale (17-item version) for the assessment of depressive symptoms. We found no associations between LPA levels (CSF or plasma) and either diagnosis or severity of MDD, or with psychotropic medication. In conclusion, our data suggest that LPA levels likely would not serve as a practical biomarker of MDD.

1. Introduction

Major depressive disorder (MDD) is the most common psychiatric illness, and the social loss associated with MDD is extremely large (Ferrari et al., 2013). However, a biochemical marker has yet to be established for clinical purposes (Smith et al., 2013).

Lysophosphatidic acid (LPA, 1-acyl-2-sn-glycerol-3-phosphoate) is well known as a potent bioactive lipid mediator with diverse biological properties (Choi et al., 2010). It is suggested that LPA plays important roles in the central nervous system (Estivill-Torrús et al., 2013). We have previously found that repeated treatment with the selective serotonin reuptake inhibitor (SSRI) sertraline altered expression of LPA-downstream genes and an LPA-related gene in rodent brain. Specifically, the levels of Rho-kinase, LIM-kinase 2, and cofilin were decreased, while the levels of Rhotekin and plasticity-related gene 1 were increased (Yamada and Higuchi, 2002). Rho/Rho-kinase signaling pathway, which is implicated in a variety of biological responses through its downstream effectors and is activated by LPA. Rho-kinase is one of the Rho effectors that

phosphorylates LIM-kinase, leading to cofilin phosphorylation. Phosphorylated cofilin modulates F-actin stabilization (Maekawa et al., 1999). We have previously reported that Rhotekin, another effector of Rho, mediates the differentiation of neuronal stem cells into neurons (Iwai et al., 2012). Plasticity-related gene 1 has been reported to increase extracellular LPA breakdown and attenuate LPA induced axonal retraction in the neuronal cells (Brauer et al., 2003). These results led us to hypothesize that LPA signaling cascades in the brain is related to the pharmacological action of antidepressants.

LPA acts in multiple cell types in the brain through its specific G-protein-coupled receptors, LPA1–7 (Frisca et al., 2012). Interestingly, LPA1-receptor knockout mice were reported to show abnormalities in emotional behavior (Harrison et al., 2003). On the other hand, intracerebroventricular injection of LPA has been shown to induce emotional changes in rats (Castilla-Ortega et al., 2014) and mice (Yamada et al., 2015). More recently, a study confirmed that in LPA1 null mice, the characteristic changes in emotional responses on behavioral tests and the activation of limbic system were reduced by an antidepressant

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desipramine (Moreno-Fernandez et al., 2017). These findings suggest that LPA is an important regulator of emotional behavior in mice, and thus possibly in the pathophysiology of MDD.

Cerebrospinal fluid (CSF) is thought to reflect molecular dynamics in the brain (Strittmatter, 2013). However, information regarding LPA levels in the CSF of patients with MDD is surprisingly limited. Therefore, we tested the hypothesis that LPA levels in the CSF are associated with MDD diagnosis, severity, and psychotropic medication in the present study. Additionally, because venipuncture is the most practical method to collect bio-samples from patients in the daily clinical setting, we also examined the same relationships using plasma samples.

2. Materials and methods

This study was conducted in accordance with the Declaration of Helsinki. The ethics committee of the National Center of Neurology and Psychiatry (NCNP) approved this study. Written informed consent was obtained from all participants.

2.1. Participants

All the patients with MDD were recruited at NCNP Hospital (Tokyo, Japan). Control participants were recruited from the community through an NCNP-biobank website announcement as well as advertisements in a local free magazine. A consensus diagnosis was made by trained psychologists or psychiatrists with DSM-IV criteria (American Psychiatric Association, 1995). We used the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I) (Otsubo et al., 2005; Sheehan et al., 1998). We excluded the participants from this study if they had a history of central nervous system disease, severe head injury, or substance abuse. Depressive symptoms were assessed by experienced research psychiatrists. We used the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (Hamilton, 1967). This scale has been shown to have good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007).

2.2. Lumbar puncture and venipuncture

Lumbar puncture was performed with the participant in the left decubitus position and CSF was withdrawn from the L3–L4 or L4–L5 interspace (Hattori et al., 2015). After removing 2 mL of CSF, an additionally 6 mL was collected and immediately transferred on ice to be centrifuged at 4 °C and aliquoted for storage at −80 °C until use. Blood samples were collected by venipuncture and plasma samples were obtained by centrifugation using standard procedures (Hattori et al., 2015).

2.3. Measurement of LPA levels

IN this study, we used Enzyme-Linked ImmunoSorbent Assay (ELISA) to quantify LPA levels. The assay kits (LPA Assay Kit II; K-2800S) were purchased from Echelon Biosciences (Salt Lake City, UT). The experiments were performed using 6 μl of CSF and 6 μl of plasma according to standard protocols. Estimation of LPA concentration in the sample was conducted using a calibration curve with GraphPad Prism 7.03 (Graph-Pad Software Inc., La Jolla, CA), according to the standard protocol.

2.4. Statistical analysis

Statistical differences between groups were calculated using one-way analysis of variance (ANOVA) or the Tukey-Kramer HSD test. Correlations were assessed using linear regression analysis. The statistical significance of differences between two groups was assessed by Student's *t*-test. The relationships between LPA levels and HAMD-17 scores were assessed by Spearman's rank correlation coefficient. Statistical analyses were performed using the JMP 12.0 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance.

Table 1
Demographics of participants.

	CSF			Plasma		
	NC	MDD	*P*	NC	MDD	*P*
N	49	52		44	47	
male	25	28		19	24	
unmedicated patients		12			9	
age (year)	41.5 (11.6)	41.9 (8.8)	0.866	40.7 (9.4)	40.6 (8.4)	0.968
body mass (kg)	62.0 (14.3)	63.1 (12.5)	0.666	63.5 (16.4)	62.0 (13.2)	0.611
BMI	22.5 (3.8)	22.8 (3.4)	0.596	23.1 (4.2)	22.6 (4.2)	0.589
sampling time (h:m)	12:34 (1:58)	13:14 (1:31)	0.058	13:22 (1:54)	12:55 (2:02)	0.257
fasting time (min)	211.8 (260.0)	135.8 (161.6)	0.138	242.9 (248.7)	403.6 (417.0)	0.033
storage time (year)	3.9 (1.0)	4.2 (1.0)	0.153	2.6 (0.8)	2.7 (1.2)	0.732
HAMD-17 score		13.4 (7.6)			16.3 (8.8)	

Values are shown as mean (standard deviation). NC: norml control, MDD: major depressive disorder. HAMD-17: 17 item Hamilton Rating Scale for Depression. The statistical significance of differences was assessed by Student's t-test.

Table 2
Comparison of average values of LPA concentrations by diagnosis.

	Diagnosis	N	Average (SD)	P	R2
CSF	NC	49	0.189 (0.077)	0.385	0.008
	MDD	52	0.175 (0.083)		
Plasma	NC	44	0.131 (0.067)	0.617	0.003
	MDD	47	0.123 (0.084)		

Values are shown as mean (standard deviation). NC: norml control, MDD: major depressive disorder. The statistical significance of differences between two groups was assessed by Student's t-test.

3. Results

The participants for CSF study were 52 patients with MDD and 49 normal healthy controls. Demographics of the participants are summarized in Table 1. All healthy controls and 12 patients with MDD were untreated with psychotropic drugs. There were no significant differences in sex ratio, age distribution, body mass, body mass index (BMI), the times of sampling, the fasting time or the storage times among groups.

The participants for plasma study were 47 patients with MDD and 44 normal healthy controls. Demographics of the participants are summarized in Table 1. All healthy controls and 9 patients with MDD were untreated with psychotropic drugs. There were no significant differences in sex ratio, age distribution, body mass, BMI, the times of sampling, or the storage times among groups. On the other hand, the fasting time was significantly longer in the patients (P = 0.033).

For both groups, we quantified LPA levels form the CSF and plasma. All data for control participants were identical to those recently reported for schizophrenia study by our group (Gotoh et al., 2019). LPA concentration did not correlate with age, although levels in female plasma were significantly higher than in male plasma (P = 0.010s, data not shown). Table 2 shows the CSF and plasma LPA levels for each group. One-way ANOVA using LPA level as the dependent variable revealed no differences between groups for either CSF or plasma samples. Table 3 shows the comparison of LPA levels by drug treatment status (medicated *vs.* un-medicated) in patients with MDD. LPA levels did not differ depending on drug treatment status in either the CSF or plasma samples.

Fig. 1 shows the correlation between CSF or plasma LPA levels and MDD severity. In this study, a significant correlation was not demonstrated between CSF LPA levels and HAMD-17 scores (P = 0.203, rs =

Table 3
Comparison of average values of LPA concentrations by drug treatment of MDD.

	Diagnosis	N	Average (SD)	ANOVA			Tukey-Kramer (P values)		
				F	P	R2	NC/Med	NC/Un-Med	Med/Un-Med
CSF	NC	49	0.189 (0.077)	F(2,98) = 1.282	0.282	0.025	0.401	0.866	0.377
	Medicated MDD	40	0.167 (0.087)						
	Unmedicated MDD	12	0.202 (0.061)						
Plasma	NC	44	0.131 (0.067)	F(2,88) = 0.390	0.678	0.009	0.760	0.949	0.748
	Medicated MDD	38	0.119 (0.076)						
	Unmedicated MDD	9	0.140 (0.118)						

Values are shown as mean (standard deviation). NC: norml control, MDD: major depressive disorder, Med: medicated MDD, Un-med: Unmedicated MDD.

0.181). A significant positive correlation was demonstrated only in females ($P = 0.045$, rs = 0.421). On the other hand, a significant correlation was not demonstrated between plasma LPA levels and HAMD-17 scores ($P = 0.218$, rs = 0.185). Analysis revealed no significant correlation in either sex.

4. Discussion

This is the first report that quantified levels of LPA in CSF and plasma samples which were obtained from patients with MDD. First, we demonstrated that LPA levels in plasma were significantly higher in females than in males. This finding is consistent with a previous report (Baker et al., 2001). However, we found that neither measure of LPA depended on age.

A meta-analysis has clearly demonstrated that plasma LPA levels are significantly higher in patients with ovarian cancer than in controls, and plasma LPA levels may thus be used as a biomarker of ovarian cancer (Li et al., 2015). In the present study, we investigated whether LPA levels could serve as a diagnostic indicator for MDD. The diagnosis of MDD largely depends on the clinical interview, and an established biochemical
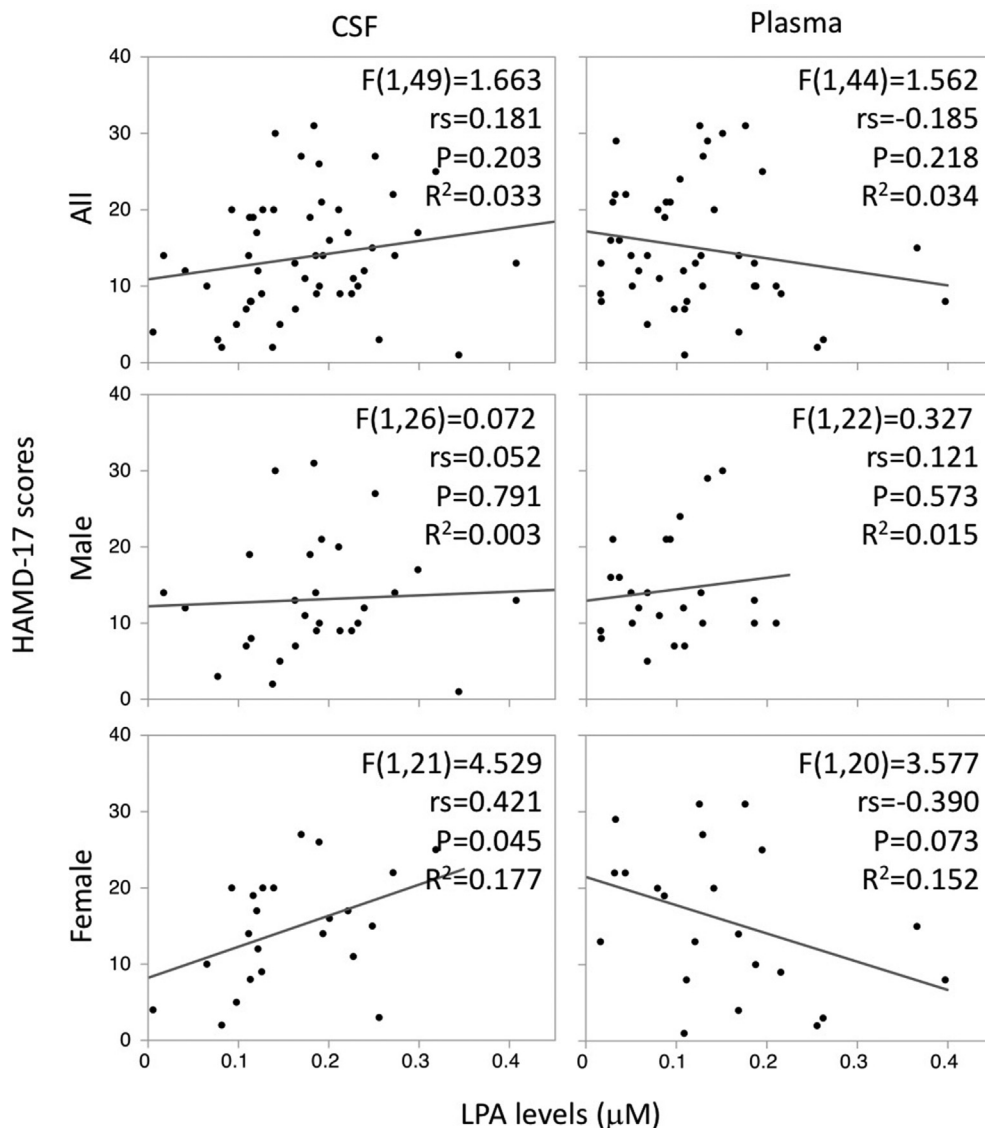

Fig. 1. Correlation between HAMD-17 scores and lysophosphatidic acid (LPA) levels in CSF and plasma with MDD patients. The relationships between LPA levels in CSF and plasma samples and HAMD scores in patients with MDD were assessed by Spearman's rank correlation coefficient. Spearman's coefficients are denoted by rs.

marker is not yet available for everyday use in the clinical setting (Smith et al., 2013). Although an omics approach seems promising, lipid mediators, such as LPA, are not the focus of transcriptomics or proteomics approaches. Therefore, here we tested the hypothesis that LPA levels in CSF are associated with a diagnosis of MDD. Despite the logic of our hypothesis, we found that LPA levels did not differ between the patients and healthy controls, and thus are unlikely to serve as a biomarker in the diagnosis of MDD. Further, patient LPA levels did not appear to differ depending on drug treatment status (medicated *vs.* un-medicated), suggesting that our results were not masked by the influence of medication. Unfortunately, we could not obtain additional data about medication from the biobank.

Finally, we examined the correlation between HAMD score (i.e., MDD severity) and LPA levels. We could not find relationship between LPA levels and MDD severity in either sample. Although a significant positive correlation was demonstrated between CSF LPA levels and HAMD-17 scores only in females, our results suggest that LPA levels (CSF or plasma) would not serve as a practical biomarker for symptomatic assessment of MDD.

This study has several limitations. The numbers of participants in the groups was rather small, particularly the numbers of medication-free patients with MDD. We need to replicate the study with a much larger sample to confirm our findings. In addition, because this study is a cross-sectional study, we are planning to use prospective sampling in our future studies to examine the relationship between the LPA signaling system and antidepressant medication in patients.

In conclusion, we found that the LPA level, either in the CSF or in plasma, would not serve as a biomarker in the diagnosis of MDD.

Declarations

Author contribution statement

Leo Gotoh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Misa Yamada: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Kotaro Hattori, Daimei Sasayama, Takamasa Noda, Sumiko Yoshida, Hiroshi Kunugi: Contributed reagents, materials, analysis tools or data.

Mitsuhiko Yamada: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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