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Increased circulating plasma lysophosphatidic acid in patients with acute coronary syndrome

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ABSTRACT

Background: The platelet activator lysophosphatidic acid (LPA) has recently been identified as an ingredient in oxidized LDL and it has been isolated from atherosclerotic plaques. The lysophospholipase D activity of autotaxin produces LPA extracellularly from lysophosphatidylcholine (LPC). The present study determines whether circulating LPA is associated with acute coronary syndrome (ACS).
Methods: We enrolled 141 consecutive patients (age, 62.6 ± 3.8 y; male, 69.2%) with ACS (n = 38), stable angina pectoris (SAP; n = 72) or angiographically normal coronary arteries (NCA; n = 31). The relationships between LPA and other established biomarkers were examined. Concentrations of plasma LPA were determined using an enzymatic assay.
Results: Concentrations of LPA significantly correlated with LPC (r = 0.549), autotaxin (r = 0.370) and LDL-C (r = 0.307) (all $p<0.01$). Lysophosphatidic acid concentrations were significantly higher in patients with ACS than with SAP and NCA ($p<0.01$), but did not significantly differ between patients with SAP and NCA. Multivariate logistic regression analyses revealed that the highest LPA tertile was independently associated with ACS (odds ratio 1.99, 95% CI: 1.18–3.39, p = 0.02).
Conclusions: The present study demonstrated that increased circulating plasma LPA concentrations are significantly associated with ACS.

1. Introduction

Lysophosphatidic acid (LPA), although originally viewed as a key intermediate in de novo lipid synthesis, has emerged as an important lipid mediator with various biological activities, which is especially important in the area of vascular biology [1–3]. Atherogenic oxidized low-density lipoprotein (LDL) contains lysophosphatidylcholine (LPC) that serves as a substrate for the production of LPA by autotaxin (lysophospholipase D, LysoPLD) [4]. In addition, LPA, which is a platelet activator and has highly thrombogenic lipid constituent of plaque, accumulates in the lipid core of human atherosclerotic lesions [5]. The platelet-activating effect of the lipid-rich core of atherosclerotic plaques and LPA involvement in this effect has been characterized [6]. Moreover, individual platelet responses to LPA might be influenced by factors that affect the degree of systemic platelet activation, such as vascular disease and blood coagulation [7–9]. Hence, an LPA receptor blockade should be a promising new approach to reducing the risk of thrombosis associated with plaque rupture [10]. We therefore considered that these findings reflect a relationship between plasma LPA concentrations and acute coronary syndrome (ACS) because of the pathophysiology associated with plaque instability and platelet aggregation. However, a relationship between LPA and other established biomarkers in patients with ACS has not been examined.

2. Methods

2.1. Study design and patient population

The present study is a prospective cross-sectional study of consecutive patients who underwent coronary angiography at Juntendo University Hospital (J-Bacchus trial) between July and December 2009. The entry criteria were as follows: no previous examination by coronary angiography, no history of coronary intervention or coronary artery bypass grafting, and having precisely evaluable coronary trees. Patients without significant stenosis according to coronary angiography were placed in a group with normal coronary arteries (NCA), whereas those with significant stenosis were defined as having coronary artery disease and placed in groups with ACS or stable angina pectoris (SAP). Patients

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with acute myocardial infarction (AMI) and unstable angina (UAP) were included in the ACS group. The diagnostic criteria for AMI and UAP were taken from the respective guidelines published by the American College of Cardiology/American Heart Association in 2007 [11,12]. These definitions of ACS depended on the specific characteristics of each element of the clinical presentation, electrocardiographic changes and a marker with high specificity for cardiac injury. Stable angina pectoris was defined as effort angina with a stable profile of symptoms for at least 3 months before admission. Demographic characteristics, medical history and current medications were determined for all participants at the time of enrollment.

We initially screened 158 patients for this study. The exclusion criteria were as follows: 1) maintenance dialysis (n = 5), 2) diabetes treated with insulin (n = 4), and 3) acute or chronic infectious (n = 5) or 4) neoplastic (n = 3) diseases. The Ethics Review Committee at our institution approved the study, all participants signed informed consent forms and the study was registered in the UMIN protocol registration system (#UMIN000002103).

2.2. Evaluation of coronary artery disease and renal function

We evaluated the severity of CAD by standard coronary angiography. All angiograms were prospectively evaluated at our angiographic core laboratory. Two expert interventional cardiologists reviewed the angiograms with no knowledge of the biomarker concentrations and patient characteristics. Disagreement over lesion characteristics was resolved by a third expert. Angiographically significant lesions were defined as >50% stenosis in vessels with a diameter ≥2.0 mm. Extensions of coronary artery disease were classified in the standard manner as 1-, 2- or 3-vessel disease.

We evaluated renal function using the estimated glomerular filtration rate (eGFR) based on the new equation published in the Japanese National Kidney Foundation guidelines [13]. The formula is as follows: $eGFR = 194 \times SCr^{-1.094} \times age^{-0.287}$, where age is in years, serum creatinine (SCr) is in mg/dl, and GFR is in ml/min per 1.73 m^2 body surface area. The product of this equation was multiplied by a correction factor of 0.739 in women.

2.3. Blood sampling and laboratory measurements

Arterial blood samples were obtained using a syringe and 18-gauge needles from the arterial sheaths of all patients before they were examined by coronary angiography in the operating room. Blood samples were directly collected into glass vacutainer tubes with or without EDTA to obtain plasma and serum, respectively. The samples were immediately placed on ice. The anticoagulated samples were centrifuged at 1000 × *g* for 10 min and then the supernatant comprising plasma was carefully collected to avoid contamination of cell components. Whole blood samples collected without EDTA-2Na were left to clot and then serum was separated by centrifugation at 1000 × *g* for 10 min.

Concentrations of plasma LPA and LPC were determined using an enzymatic assay as described [14–16]. In brief, LPA was hydrolyzed with lysophospholipase to glycerol 3-phosphate, followed by enzymatic cycling using glycerol 3-phosphate oxidase and glycerol 3-phosphate dehydrogenase. The amplified concentrations of hydrogen peroxide, a product of enzymatic cycling, were then colorimetrically measured (JCA-BM8040, JEOL, Tokyo, Japan). Lysophosphatidylcholine concentrations in human plasma were measured using our validated enzymatic assay [16] in which LPC is converted by lysophospholipase into glycerophosphophorylcholine, from which glycerophosphorylcholine phosphodiesterase generates choline. The hydrogen peroxide produced from choline by choline oxidase was determined in the presence of peroxidase using an oxidative chromogenic reagent and 4-aminoantipyrine by measuring changes in absorbance. Serum highly sensitive C-reactive protein (hs-CRP) was measured using a validated, highly sensitive immunoassay. The activity of lipoprotein associated phospholipase A2 (Lp-PLA2) in serum was spectrophotometrically assayed as described [17]. Concentrations of serum cardiac troponin T were measured using a chemiluminescent enzyme immunoassay kit (Determiner CL TnT, Kyowa Medex, Tokyo, Japan). Serum autotaxin was quantified using a 2-site immunoenzymetric assay as described [18]. Other markers were determined by routine laboratory methods.

2.4. Statistical analysis

All data were statistically analyzed using SPSS ver. 18.0 (Chicago, IL) and JMP ver. 7.0 (SAS Institute Inc., Cary, NC). The distribution of continuous variables was assessed by visual inspection of frequency histograms and using the Shapiro–Wilk test. Results are presented as medians and inter-quartile ranges (IQR), median ± standard deviation or as ratios (%) and numbers for categorical data. Values obtained from three groups were compared by the one-way analysis of variance (ANOVA), the Kruskal–Wallis test and the χ^2 analysis. The post-hoc Scheffé test compared parameters within groups. Because of the known association between LPA and the other markers, natural log transformation of the LPA data achieved a normal distribution, and thus log-transformed LPA values were used in this study. Correlations were searched using Spearman's rank correlation. The independent effect of the biomarkers on the risk of ACS adjusting for potential confounders was determined using multiple logistic regression analysis. We evaluated the effect of the biomarkers Lp-PLA2, hs-CRP, and LPA in this model according to tertile increments in the concentrations of each. The following variables were initially incorporated into the univariate model: age, sex, diabetes, dyslipidemia, current smoking, angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB), statins, HbA1c, eGFR and tertiles of Lp-PLA2, hs-CRP, and LPA. Variables with p values of <0.20 were then entered into the multivariable model. A $p<0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of study participants

We enrolled 141 patients (age 65.8 ± 11.5 y, male sex 78.0%), all of whom had angiographically documented coronary trees with confirmed clinical features, and a diagnosis of NCA (n = 32, 22.7%), SAP (n = 71, 50.4%) and ACS (n = 38, 27.0%; UAP = 17, AMI = 21). Table 1 shows the baseline characteristics of the three groups. Briefly, the three groups were similar with respect to age and body mass index but differed in terms of cardiovascular risk factors, which were more frequent in patients with SAP and ACS than with NCA. The patients with ACS tended to have lower eGFR concentrations than the NCA and SAP groups. Moreover, patients with SAP more frequently received cardiovascular therapy than those with NCA and ACS.

3.2. Plasma LPA concentrations

Circulating plasma LPA concentrations did not significantly differ between males and females (median: 0.375 vs. 0.47 μmol/l, p = 0.102), or between those with or without conventional risk factors such as diabetes (median: 0.36 vs. 0.41 μmol/l, p = 0.408), current smoking (median: 0.365 vs. 0.42 μmol/l, p = 0.156), dyslipidemia (median: 0.37 vs. 0.435 μmol/l, p = 0.125) or hypertension (median: 0.38 vs. 0.435 μmol/l, p = 0.174). The concentration of autotaxin, which produces LPA from LPC through its lysoPLD activity, was higher in females than in males (median: 0.90 vs. 0.65 mg/l, p < 0.001) [18].