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Increased lysophosphatidic acid levels in culprit coronary arteries of patients with acute coronary syndrome

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ABSTRACT

Background: Lysophosphatidic acid (LPA) is a platelet activator and highly thrombogenic lipid constituent of atherosclerotic plaque. However, whether or not LPA locally released from culprit lesions is associated with acute coronary syndrome (ACS) remains unclear.
Methods: We studied 52 patients with ACS who were treated by emergency percutaneous coronary intervention and thrombectomy. Levels of LPA and other established biomarkers were enzymatically assayed in samples of culprit coronary arterial and systemic peripheral arterial blood. Levels of LPA and lysophosphatidylcholine (LPC) were measured in plasma, and those of autotaxin, soluble CD40 ligand (sCD40L), hs-CRP and Lp-PLA2 were measured in serum.
Results: Median LPA levels were significantly higher in coronary (CB) than in peripheral (PB) arterial blood ($p = 0.009$). Levels of sCD40L were higher in CB than in PB, but the difference did not reach statistical significance ($p = 0.177$). In contrast, autotaxin and Lp-PLA2 levels were significantly higher in PB than in CB ($p = 0.005$ and $p = 0.038$, respectively). Levels of LPC and hs-CRP were also higher in PB than in CB ($p = 0.129$ and $p = 0.121$, respectively). Levels of LPA in both CB and PB were positively and significantly associated with those of LPC ($r = 0.632$, $p < 0.01$ and $r = 0.465$, $p < 0.001$).
Conclusions: Culprit coronary arteries of ACS contained significantly more LPA than the systemic arterial circulation. Higher LPA concentrations might be associated with the pathophysiology of ACS.

1. Introduction

Lysophosphatidic acid (LPA) has emerged as an important lipid mediator with various biological activities that are particularly important to vascular biology [1–3]. Accumulating evidence indicates that LPA plays several biological roles related to blood cells and cells of vessel walls (endothelial cells, smooth muscle cells), which are all key players in atherosclerotic and atherothrombotic processes [4,5]. Atherogenic oxidized low-density lipoprotein (LDL) contains lysophosphatidylcholine (LPC) that serves as a substrate for the production of LPA by autotaxin (lysophospholipase D, LysoPLD) [6]. The production of LPA is also associated with changes in atherosclerotic plaque formation. The increased deposition of potent platelet-activating and proinflammatory LPC species in advanced atherosclerotic lesions indicates that thin cap fibroatheromas can be characterized not only by cellular and morphological features, but also by their prothrombotic lipid profiles [7]. In addition, LPA is abundant in the lipid-rich core of human atherosclerotic plaque lesions [8]. After plaque rupture or erosion, exposure to LPA in the lipid-rich core might play a key role in triggering or potentiating platelet responses during acute thrombosis [9,10]. Our previous cross-sectional study found significantly higher concentrations of circulating plasma LPA in patients with acute coronary syndrome (ACS) than in those with stable angina or normal coronary arteries [11].

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However, clinical trials have not uncovered significant evidence of a mechanism of LPA production or the circulatory status of LPA in pathophysiological ACS. In particular, little is known about an association between LPA and plaque rupture during ACS. We therefore determined the clinical significance of LPA levels using coronary catheter intervention in patients with ACS and coronary plaque instability, and examined relationships between LPA and other established biomarkers.

2. Methods

2.1. Study participants

This prospective cross-sectional study comprised 52 consecutive patients with ACS who underwent emergency PCI and thrombectomy at Juntendo University Hospital between January and December 2009. The entry criteria were as follows: no history of coronary intervention or coronary artery bypass grafting and having evaluable coronary trees. Information about demographics, medical history and current medications was collected from all participants at the time of enrollment. The diagnosis of ACS was determined according to the 2007 American College of Cardiology/American Heart Association criteria [12,13]. The definition of ACS depended on the specific characteristics of individual elements of each clinical presentation, electrocardiographic findings and highly specific markers of cardiac damage.

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. Study protocol

We collected all local blood samples in the operating room at the time of emergency coronary angiography. Patients received standard medication for ACS that comprised aspirin (162 mg) and clopidogrel (300 mg). Heparin (100 IU/Kg) was administered after identifying culprit lesions. A 0.014-inch guide wire was then advanced across the culprit lesion, and a thrombectomy catheter (Thrombuster, Kaneka Medics, Osaka, Japan) was inserted through the guiding catheter. A 20-cc syringe was used to withdraw both coronary blood (CB) from the culprit coronary artery through the thrombectomy catheter and peripheral blood (PB) from the arterial sheath (6-Fr or 7-Fr). Samples were then transferred using an 18-gauge needle into glass vacutainer tubes with or without EDTA to obtain plasma and serum, respectively, and immediately placed on ice. The anticoagulated samples were centrifuged at 2500× *g* for 30 min at 4 °C, and then the plasma supernatant was carefully decanted to avoid contamination with cell components. Samples of whole blood sample were left at room temperature for 15 min to allow blood clots to form, and then the serum was separated by centrifugation at 2500× *g* for 30 min at 4 °C. All samples were stored at −80 °C.

2.3. Laboratory measurements

We measure plasma levels of LPA and LPC, and serum levels of autotaxin (ATX), soluble CD40 ligand (sCD40L), hs-CRP, and Lp-PLA2.

Plasma LPA and LPC concentrations were enzymatically determined 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 using an automatic analyzer (JCA-BM8040, JEOL, Tokyo, Japan). Lysophosphatidylcholine concentrations in human plasma were measured using our validated enzymatic assay in which LPC is converted by lysophospholipase into glycerophosphorylcholine, from which glycerophosphorylcholine phosphodiesterase generates choline. The amount of 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 ATX antigen concentrations were determined using a specific sandwich enzyme immunoassay that has proven useful for clinical laboratory testing [17]. Serum ATX antigen concentrations are associated with both plasma LPA concentration and serum lysoPLD activity, which is responsible for LPA production [18]. Serum hs-CRP was measured using a validated, highly sensitive immunoassay and particle-enhanced immunonephelometry (Dade Behring Holding GmbH, Liederbach, Germany). We quantified sCD40L using a human sCD40L ELISA kit (R&D Systems, Minneapolis, MN, USA). Serum Lp-PLA2 activity was determined spectrophotometrically as described [19]. Levels of serum cardiac troponin T were measured using a chemiluminescent enzyme immunoassay kit (Determiner CL TnT, Kyowa Medex, Tokyo, Japan). Other markers were routinely determined.

2.4. Statistical analysis

All data were statistically analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and JMP version 9.0 (SAS Institute Inc., Cary, NC, USA). Results are presented as medians and inter-quartile ranges (IQR), means ± standard deviation or as ratios (%) and numbers for categorical data. Data from pairs were compared using the Wilcoxon rank-sum test. Because of known differences between coronary and systemic alterations in biomarker levels, the natural log transformation of the biomarker data achieved a normal distribution, and thus we used log-transformed marker values. Correlations were searched using Spearman's rank correlation. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of study participants

Table 1 shows the characteristics of the 52 patients (male, 65%; mean age, 63.2 ± 13.6 years), 65% ($n = 34$) of whom had ST elevation myocardial infarction (STEMI). The left anterior descending artery was the culprit coronary artery in >50% of the patients. All patients received aspirin and clopidogrel, and most were also taking β-blockers at the time of blood collection, before undergoing emergency percutaneous coronary intervention and thrombectomy. Four patients had cardiogenic shock, so hemodynamic support was provided using intraaortic balloon pumping (IABP).

3.2. Comparison between coronary circulation and systemic circulation

Only median LPA levels were significantly higher in culprit CB than in PB (0.266 [IQR, 0.192–0.300] vs. 0.230 [IQR, 0.180–0.287] μM, $p = 0.009$). Median sCD40L levels were higher in CB than in PB, but the difference did not reach statistical significance (2055.0 [IQR, 1017.5–3375.0] vs. 1410.0 [IQR, 874.0–2485.0] pg/mL, $p = 0.177$) In contrast, autotaxin and Lp-PLA2 levels were significantly higher in PB than in CB (autotaxin: 0.671 [IQR, 0.589–0.837] vs. 0.632 [IQR, 0.496–0.738] mg/L, $p = 0.005$; Lp-PLA2: 442.0 [IQR, 347.0–556.5] vs. 436.5 [IQR, 318.8–537.3] IU/L, $p = 0.038$). In addition, LPC and hs-CRP levels were higher in PB than in CB (LPC: 163.0 [IQR, 133.5–187.5] vs. 158.0 [IQR, 119.8–191.8] μM, $p = 0.129$; hs-CRP: 1235 [IQR, 435–3250] vs. 993 [IQR, 395–2845] ng/mL, $p = 0.121$) (Fig. 1).