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Analysis of Plasma and Hematoma Lipids Related to Choline Glycerophospholipid in Patients with Chronic Subdural Hematoma

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Abstract

The levels of platelet-activating factor (PAF) and lipid metabolites related to choline glycerophospholipid were measured in the plasma and hematoma samples obtained from patients with chronic subdural hematoma. The ratio of lyso-choline glycerophospholipids (lysoPC) to choline glycerophospholipids (PC) in hematoma correlated with the interval between the onset of symptoms and surgery. PC and lysoPC fatty acyl moieties in plasma and hematoma were essentially similar. These results suggest that the lysoPC to PC ratio in hematoma can determine the age of the chronic subdural hematoma, and that the origin of hematoma may be circulating blood. The levels of PAF in the plasma of chronic subdural hematoma patients were significantly greater than in healthy volunteers. PAF may be involved in the enlargement of chronic subdural hematoma.

Key words: chronic subdural hematoma, platelet-activating factor, lyso-platelet-activating factor, choline glycerophospholipid, lyso-choline glycerophospholipid

Introduction

The etiological factors in the enlargement of chronic subdural hematoma have not yet been established, although the rebleeding hypothesis of Bergström *et al.*[2] is widely accepted, which suggests that recurrent hemorrhage into hematoma cavity from sinusoidal channels in the capsule contributes to the enlargement of the hematoma. Ito *et al.*[9] demonstrated local hyperfibrinolysis in the outer membrane of chronic subdural hematoma. Such local hyperfibrinolysis may also participate in the mechanism of enlargement of hematoma.

The presence of chronic subdural hematoma may stimulate continuous inflammation. Platelet-activating factor (PAF) from the broken blood cells in the hematoma[1,5,6,10,11,13,16] may induce the chemotaxis of inflammatory cells to the capsule.[20,21,24] PAF can also stimulate the synthesis and release of tissue plasminogen activator (t-PA) in endothelial cells.[17] Therefore, PAF may be one of the mediators which provoke the enlargement of chronic subdural hematoma. The increased vascular permeability induced by PAF may also contribute to rebleeding from the vessels of the hematoma capsule.[3]

Choline glycerophospholipid (PC) is hydrolyzed to lysoPC by phospholipase A_2. PAF is synthesized from one subclass of lysoPC, lysoPAF, by transacetylation. PAF is metabolized to lysoPAF by PAF acetylhydrolase. These lipids are all closely linked metabolically.

The present study therefore analyzed PAF and other lipid metabolites related to PC in the plasma and hematoma of patients to investigate possible involvement in the mechanism of enlargement of chronic subdural hematoma.

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Patients and Methods

This study examined surgical samples of 17 chronic subdural hematomas and 14 plasma samples from 14 patients, 11 males and three females aged 48–88 years (median 71.4 yrs). Three patients had bilateral

Table 1 Summary of 14 cases of chronic subdural hematoma

Case No.	Age/ Sex	Trauma	Symptoms	Interval from the onset of symptoms to operation (day)	Side(s) of hematoma
1	67/M	+	headache, gait disturbance	5	lt
2	65/M	+	aphasia, rt hemiparesis	14	lt
3	55/M	+	dementia	40	lt
4	88/F	+	gait disturbance	60	lt
5	48/M	+	headache, gait disturbance	1	bil
6	69/M	+	gait disturbance	6	bil
7	62/M	+	headache, vomiting	14	bil
8	84/M	+	vertigo, headache	30	lt
9	80/M	+	gait disturbance	20	lt
10	66/M	+	headache, gait disturbance	14	rt
11	77/M	+	gait disturbance	10	lt
12	86/F	+	dizziness	45	lt
13	75/F	+	gait disturbance	60	rt
14	78/M	+	dementia	60	lt

hematomas (Table 1). All 17 hematomas were confirmed at surgery to have complete capsules with both inner and outer membranes. Normal PAF levels in plasma were measured in plasma specimens from seven healthy male volunteers aged 41–76 years (median 64.5 yrs). Coagulation was prevented in plasma samples by adding 3.8% sodium citrate solution in a 1:9 ratio. Plasma and hematoma samples were centrifuged at 1100 *g* for 10 minutes at room temperature. The soluble fraction was used for analysis. All specimens were frozen at −80°C until lipid analysis.

Brain computed tomographic (CT) scans were taken of the patients on the day of surgical treatment and the CT number of the hematoma measured. The mean CT number of hematomas was calculated in patients with bilateral hematomas.

Total lipids were extracted from both hematoma and plasma specimens by the method of Bligh and Dyer.[4] Extracts were applied to a silica gel cartridge (Sep-pak®; Waters, Milford, Mass., U.S.A.) which was washed with 10 ml of chloroform, 10 ml of acetone, 10 ml of an acetone and methanol mixture (1/1 v/v), and 10 ml of a chloroform and methanol mixture (7/3 v/v). The elute, obtained with 10 ml of a chloroform, methanol, and water mixture (1/2/0.8 v/v/v), was collected and extracted.[4] The lipid extract was separated by thin-layer chromatography (Silicagel G; Merck, Darmstadt, Germany) using a chloroform, methanol, and water (65/35/6 v/v/v) solvent system. Areas corresponding to PC, PAF, and lysoPC were scraped off and lipids were extracted.[4] LysoPAF was separated from lysoPC by high-performance liquid chromatography with a 5uSi-100A column (150 × 8.9 mm) (LC-6A; Shimadzu, Tokyo) and an isopropanol-hexane-water system (110/100/20 v/v/v) at a flow rate of 0.4 ml/min. The lysoPAF fraction was eluted between 21.5 and 23.5 minutes, while 1-acyl-glycero-3-phosphocholine was eluted between 23.5 and 26 minutes. The lysocompound elution times were confirmed using [^{3}H]lyso-PAF and 1-[^{14}C]acyl-glycero-3-phosphocholine. The PC and lysoPC contents were determined by the method of Rouser *et al.*[18] PAF and lysoPAF were quantified by bioassay, measuring the radioactivity of [^{3}H]serotonin released from rabbit platelets.[15] LysoPAF was acetylated and added to the reaction mixture. The PC and lysoPC fatty acyl moieties were estimated by gas-liquid chromatography using 15% DEGS (60/80 mesh) (Gasukuro Kogyo Co., Tokyo). Fatty acid chains are designated by the number of carbon atoms and the number of double bonds, *e.g.* 18:1 for oleic acid.

All chemicals were of reagent grade, and solvents were distilled before use. Standard mixtures of fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). [Hexadecyl-1′,2′-^{3}H(N)]PAF and [alkyl-^{3}H(N)]lysoPAF were purchased from DuPont (NEN Research Products, Wilmington, Del., U.S.A.). 1-[1-^{14}C]palmitoyl-lysophosphatidylcholine and 5-hydroxy[side chain-2-^{14}C]tryptamine creatinine sulfate were obtained from the Radiochemical Center (Amersham, Buckinghamshire, U.K.).

Numerical values were evaluated by Student's t-test and $p < 0.05$ was defined as statistically significant.

Results

Table 2 shows the mean ± SEM of the measurements of PC, lysoPC, PAF, and lysoPAF levels in the plasma and hematoma samples from the patients with chronic subdural hematoma and healthy volunteers. Plasma PC (2 samples), lysoPC (2), and lysoPAF (2) levels, and hematoma PC (3), lysoPC (3), PAF (1), and lysoPAF (3) levels could not be measured due to the small volume of the samples.

Table 2 Lipid levels in plasma and hematoma samples

Sample group	PC (μmol/ml)	LysoPC (nmol/ml)	PAF (pmol/ml)	LysoPAF (pmol/ml)
Plasma				
control	—	—	0.18 ± 0.02 (7)	31.5 ± 30.7 (7)
patients	3.84 ± 0.72 (12)[b]	82.9 ± 18.3 (12)	2.45 ± 1.48 (14)[a]	50.7 ± 21.9 (12)
Hematoma				
patients	1.05 ± 0.24 (14)	61.8 ± 24.0 (14)	0.54 ± 0.20 (16)	93.1 ± 59.7 (14)

Values are means ± SEM. Numerals in parentheses indicate no. of measurements. [a]Significantly different from the control plasma level ($p < 0.01$). [b]Significantly different from the hematoma level ($p < 0.05$).

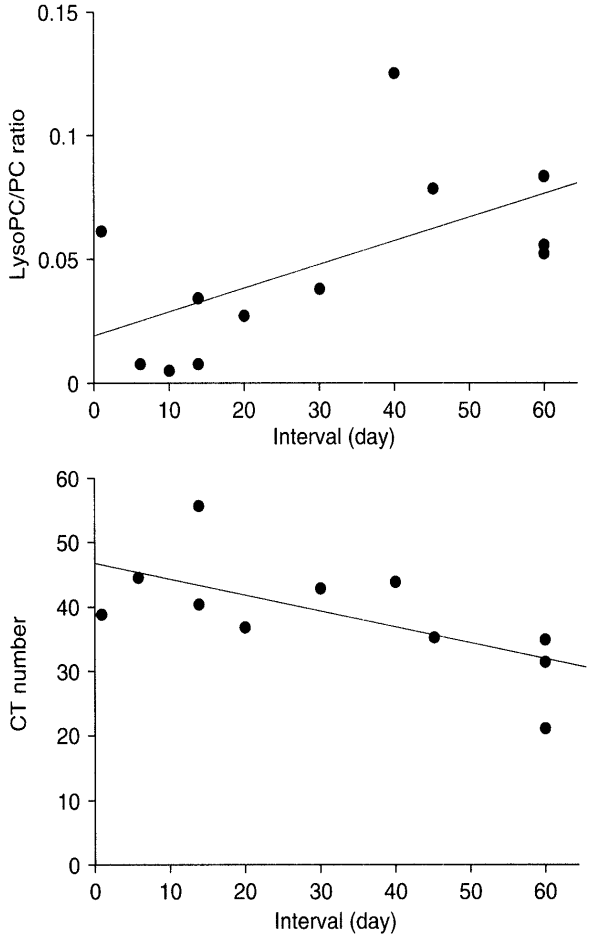

Fig. 1 Changes in lysoPC/PC ratio (*upper*: $Y = 9.4 \times 10^{-4}X + 2.11 \times 10^{-2}$, $r = 0.58$, $p < 0.05$) and CT number (*lower*: $Y = -0.27X + 47.2$, $r = -0.67$, $p < 0.05$) with interval between the onset of symptoms and operation in patients with chronic subdural hematoma.

The PC concentration was significantly ($p < 0.05$) higher in the plasma than the hematoma samples. There was no significant difference in lysoPC levels between the plasma and hematoma.

The lysoPC to PC ratio in hematoma demonstrated a significant ($p < 0.05$) correlation with the interval between the onset of symptoms and operation (Fig. 1). Likewise, the CT number of the hematoma was negatively correlated with the interval ($p < 0.05$), although the CT number in three patients could not be measured. These results suggest that older hematoma demonstrate a higher lysoPC/PC radio and a lower CT number.

Table 3 Fatty acyl chain composition of PC and lysoPC in plasma and hematoma in patients with chronic subdural hematoma

Fatty acyl chain	Plasma		Hematoma	
	PC (%)	LysoPC (%)	PC (%)	LysoPC (%)
16:0	21.4	28.7	19.8	39.2
18:0	15.4	17.2	17.4	24.6
18:1	14.0	6.4	20.3[a]	7.0
18:2	20.8	47.8	16.0	29.3
20:3	2.9	—	2.7	—
20:4	10.6	—	9.0	—
22:5	1.3	—	2.2	—
22:6	13.6	—	12.6	—

[a]Significantly different from the plasma level ($p < 0.01$).

The fatty acid chains of PC and lysoPC were similar in both hematoma and plasma, except for a higher 18:1 content in hematoma PC ($p < 0.01$) (Table 3).

Plasma levels of PAF were significantly higher in the patients than the control group ($p < 0.01$) (Table 2).

Discussion

Analysis of the PC and lysoPC fatty acid chains in plasma and hematoma samples showed that although the ratio of 18:1 in hematoma PC was higher than that in plasma, there were essentially no differences. This result suggests the origin of hematoma is circulating blood. However, we cannot

conclude whether whole blood or only the plasma fraction leaks into the hematoma cavity. Erythrocytes[7] and neutrophils[12] have a relatively higher 18:1 ratio in PC than plasma.[22] Therefore, whole blood, not just plasma, may leak into the hematoma cavity and cause the higher 18:1 ratio in hematoma.

Scotti *et al.*[19] reported the CT number in chronic subdural hematoma to negatively correlate with the interval between the onset of symptoms and the date of CT examination. We obtained the same results. Hematomas have phospholipase A (A_1 and A_2) activity due to circulating blood cells.[8,14,23] Therefore, the enzyme product, lysoPC, may accumulate in the hematoma time-dependently. To estimate the interval between rebleeding and operation, we measured the ratio of lysoPC to PC in the hematoma. The ratio correlated well with the interval between the onset of symptoms and operation. The measurement of lysoPC/PC ratio in hematoma can therefore be used to determine the age of a chronic subdural hematoma.

We found the plasma PAF levels in the patients to be higher than healthy volunteers. The hematoma PAF levels in the patients showed a tendency to be higher than healthy volunteer plasma levels. Therefore, increased systemic and local PAF may be related to rebleeding in the subdural hematoma by stimulating the synthesis and release of t-PA in endothelial cells and increasing vascular permeability.[3,17]

The high PAF level in the plasma of a chronic subdural hematoma is very interesting. Although the healing mechanism of chronic subdural hematoma is also controversial, only irrigation of hematoma induces the healing of subdural hematoma in most patients. This suggests that the presence of hematoma is a source of inflammation, or substances present in hematoma may stimulate the continuous production of mediators which provoke the enlargement of hematoma. PAF may be one of these mediators. There was no difference in the plasma and hematoma levels of lysoPAF in control and patients, suggesting that the synthesis and breakdown of PAF was relatively stable in plasma and hematoma. The high PAF level in the plasma may therefore be due to blood cells such as inflammatory cells and platelets.

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