Original

PHOSPHOLIPASE A_2 AND PROSTAGLANDIN E_2 IN CEREBROSPINAL FLUID FOLLOWING SUBARACHNOID HEMORRHAGE

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Abstract

The mechanisms leading to early brain injury and vasospasm following subarachnoid hemorrhage (SAH) remain unclear. The present study was carried out to determine the time courses of cerebrospinal fluid (CSF) and plasma, phospholipase A_2 (PLA_2) and prostaglandin E_2 (PGE_2) after SAH.

We studied 10 patients who underwent aneurysm clipping after SAH and required a drain catheter into the basal cistern for 10 days postoperatively. CSF and arterial blood were sampled every day from day 1 to day 10 postoperatively. Glasgow Coma Scale (GCS) was calculated as index of neurologic function at the sampling time. PLA_2 activities in CSF and plasma were measured by Dole assay. PGE_2 concentrations in CSF and plasma were measured by radioimmunoassay.

There was no significant change either in plasma PLA_2 activity or PGE_2 concentration after SAH. CSF PLA_2 activity and PGE_2 concentrations on days 1–3 and 1–2 in SAH patients were increased significantly compared with those during days 4–10 and 3–10, respectively. GCS on days 1–2 in SAH patients was increased significantly compared with those during days 3–10.

PLA_2 activity and PGE_2 concentration in CSF increased within 3 days after SAH. Thus, CSF PLA_2 in CSF would play a role in early brain injury, rather than the delayed cerebral vasospasm after SAH.

(JJOMT, 53 : 206–212, 2005)

—Key words—

Subarachnoid hemorrhage, phospholipase A_2, prostaglandin E_2

Introduction

Early brain injury after subarachnoid hemorrhage (SAH) determines the prognostic outcome. Major early brain injuries after SAH are breakdown of blood-brain barrier (BBB) and formation of brain edema. Although few studies have examined early brain injury after SAH, prostaglandin E_2 (PGE_2) might contribute to acute brain edema after trauma[1)2)]. Brain edema deteriorates neurologic function.

Delayed cerebral vasospasm after SAH affects patient's outcome, too. Research into the mechanisms of the delayed vasospasm has led multifactorial hypotheses, but it is generally agreed that extra luminal oxyhemoglobin released from erythrocytes plays a major role. Moreover, several studies have implicated a role of arachidonic acid metabolites[3)] including thromboxan A_2[4)], prostacyclin[5)], platelet activating factor[6)] and 20-hydroxyeicosetetraenoic acid[7)] in cerebral vasospasm. Delayed cerebral vasospasm after SAH deteriorates neurologic function, too.

It was reported that endothelins would play an important role in the pathogenesis of cerebral vasospasm after SAH, and endothelins in rat brain capillary endothelial cells activated phospholipase A_2 (PLA_2)[8)]. Free fatty acids including arachidonic acid of cerebrospinal fluid (CSF) remained elevated for the first 2 days following SAH[9)]. Second elevation of them was observed after 7 days following SAH. Although group IIA PLA_2 is a key enzyme in the production of arachidonic acid metabolites, the role and the time course of PLA_2 after SAH remain unclear.

The present study was carried out to determine the time courses of the change in CSF and plasma PLA_2 and PGE_2, and simultaneously evaluate Glasgow Coma Scale (GCS) as an index of neurologic function after SAH.

Materials and Methods

Patients

After the approval of Institutional Research Committee, an informed consent was obtained from each patient's relatives. We studied consecutive 15 patients who underwent aneurysm clipping after aneurysm-induced SAH from October 2001 to April 2002 at the Nagasaki Rosai Hospital in Sasebo, Japan. The study's exclusion criteria included the following conditions: a) removal of a ventricular catheter within 10 days postoperatively, b) sepsis, and c) re-operation. Ten patients of these 15 patients were eligible for inclusion in the study. A ruptured aneurysm was clipped within 24hrs of the ictus and a drain catheter was placed into the basal cistern. CSF was intermittently drained to maintain the intracranial pressure below 20 mmHg. After the operation, conventional hypervolemic hemodilution and induced hypertension were applied for prevention and treatment of SAH-induced cerebral vasospasm. All patients received conventional brain-oriented intensive care therapy according to clinical requirements. Symptomatic cerebral vasospasm was defined with neurological deficits and abnormal transcranial Doppler sonography. The outcome was assessed with the Glasgow Outcome Scale at the discharge from hospital.

Arterial blood and cisternal CSF were sampled for the measurement of PLA_2 activity and PGE_2 concentration in the every morning from day 1 to day 10 after the operation. The GCS was also calculated for each patient at the sampling time.

PLA_2 Analysis

Samples were centrifuged at 3,000 rpm for 10 min. Plasma was diluted 50 fold in buffer consisting of 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA and protease inhibitors consisting 20 μM leupeptin and 0.1 mM phenylmethyl sulfonyl fluoride (PMSF). CSF was added the protease inhibitors consisting 20 μM leupeptin and 0.1 mM PMSF. Diluted plasma and CSF were stored at –80°C until assayed. PLA_2 activity was measured by the previously described method with some modifications[10)11)]. L-3-phosphatidylethanolamine, 1-acyl-2-[1-^{14}C] arachidonyl (PE) (Amersham, Buckinghamshire, UK) was used as exogenous substrate, which was dried under N_2 and resuspended in ethanol. The PLA_2 assay buffer (100 μL) contained 75 mM Tris-HCl, 10 mM $CaCl_2$, and 0.22 nmol of the PE (~25,000 cpm) at pH 9.0. The reaction was carried out at 37°C for 30 min and was stopped by adding 0.56 mL of Dole's reagent: 48.75% isopropyl alcohol, 50% n-heptane, 1.25% 1N H_2SO_4 in water. Arachidonic acid (AA) was extracted in the following manner. Water, 0.11 mL, was added and the sample was vortexed and centrifuged at 10,000 g for 5 min. A volume of 0.15 mL of the upper phase was transferred to a new tube to which 50 μL silica gel and 0.8 mL of n-heptane were added. The samples were vortexed and centrifuged again for 5 min each. A volume of 0.8 mL of supernatant was then counted in a liquid scintillation counter. PLA_2 activity was expressed as pmol of radiolabeled AA released from PE per min per mL of plasma or CSF.

PGE_2 assay

Samples were added 10^{-5}M indomethacin and centrifuged at 3,000 rpm for 10 min. Plasma and CSF were stored at –80°C until assayed. PGE_2 concentrations in CSF and plasma were measured by radioimmunoassay in duplicates using commercially available standard kits (Amersham Pharmacia Biotech, Buckinghamshire, England). Within-assay coefficients of variation were less than 6% and between-assay coefficients of variation were less than 12% for each radioimmunoassay procedure. The lower limit of sensitivity was 10 pg/mL.

Control

The control samples of arterial blood were collected from 7 patients (age 56–73, 4 females and 3 males) who underwent orthopedic surgery. After general anesthesia and cannulation into radial artery, samples were drown for PLA_2 and PGE_2 assay. The control samples of CSF were collected from 8 patients (age 48–74, 4 females and 4 males) who underwent lumbar spinal taps for spinal anesthesia. The control samples were taken once for each person.

Statistical analysis

Results were presented as mean ± SEM. Comparisons of the concentrations over time were made using the one way analysis of variance for repeated measures and student t test with $p< 0.05$ regarded as significant.

Results

In the control group, PLA_2 activity and PGE_2 concentration in CSF were 3 ± 1 pmol/min/mL and 78 ± 3 pg/mL, respectively. Plasma PLA_2 activity and PGE_2 concentration in the control group were 3 ± 1 pmol/min/mL and 198 ± 26 pg/mL, respectively. The mean age of the patients was 61 ± 3 yrs (range, 48–73). The mean GCS on admission was 13 ± 1 (range, 10–14). Three patients had a symptomatic vasospasm (Table 1). Five patients had a good recovery at discharge.

As shown in Fig. 1, there was no significant change throughout the time course either in plasma PLA_2 activity or PGE_2 concentration after SAH. As shown in Fig. 2, CSF PLA_2 activity and PGE_2 concentration on days 1–3 and 1–2 in SAH patients increased significantly compared with those during days 4–10 and 3–10, respectively. Plasma PLA_2 activity and PGE_2 concentration after SAH were similar to those in control subjects. CSF PLA_2 activity and PGE_2 concentration increased significantly compared with those in control subjects. The concentrations of PLA_2 and PGE_2 in plasma and CSF were similar between the patients with and without symptomatic vasospasm (data

Table 1 Patient Characteristics

No	age (year)	sex	A-Site	GCS	H-K	spasm	H-day (day)	GOS
1	67	F	left MC	10 (E4, V1, M5)	G4	no	152	D
2	48	M	left IC	14 (E3, V5, M6)	G3	yes	62	GR
3	73	F	right AC	10 (E3, VT, M5)	G3	no	186	MD
4	68	F	right MC	6 (E1, V1, M4)	G4	no	87	PVS
5	69	F	left PICA	14 (E3, V5, M6)	G1	no	69	GR
6	70	M	A com	13 (E3, V4, M6)	G3	no	70	SD
7	58	M	left MC	14 (E3, V5, M6)	G3	yes	91	MD
8	54	F	left IC-PC	14 (E4, V4, M6)	G3	no	64	GR
9	55	F	right MC	12 (E2, V4, M6)	G3	no	43	GR
10	51	M	right AC	14 (E4, V4, M6)	G1	yes	65	GR

Abbreviation: No, number; A-Site, Aneurysm-site; GCS, Glasgow Coma Scale on admission; H-K, Hunt and Kosnik grade on admission; spasm, vasospasm; H-day, Hospital day; GOS, Glasgow Outcome Scale at discharge; F, female; M, male; MC, middle cerebral artery; IC, internal carotid artery; AC, anterior cerebral artery; PICA, posterior inferior cerebellar artery; A com, anterior communicating artery; PC, posterior cerebral artery; E, eye opening; V, verval response; M, best motor response; D, death; GR, good recovery; MD, moderately disability; PVS, persistent vegetative state; SD, severe disability;

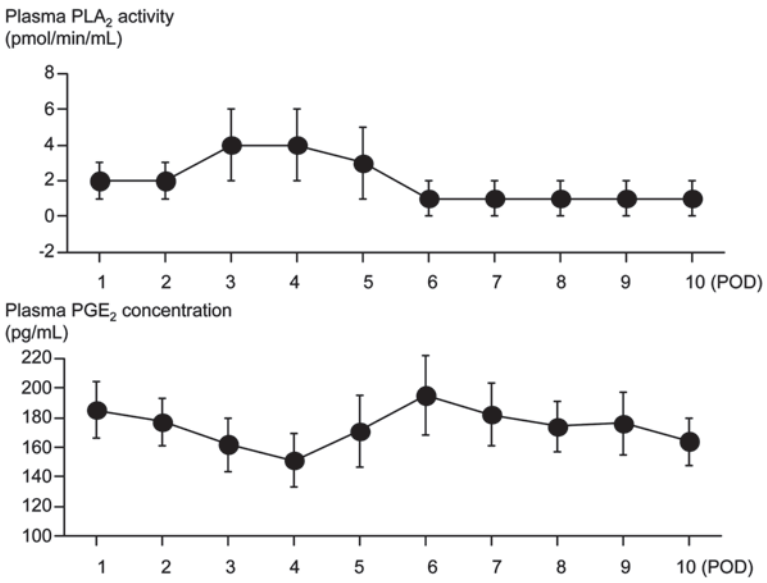

Fig. 1 The time course of plasma phospholipase A_2 (PLA_2) activity (the upper) and plasma prostaglandin E_2 (PGE_2) concentration (the lower) after subarachnoid hemorrhage. Data are expressed as mean ± SEM. # $p<0.05$ compared with value of other day.

was not shown).

As shown in Fig. 3, GCS during days 1–2 in SAH patients were decreased compared with those during days 3–10.

Discussion

The results show that the CSF PLA_2 activity and PGE_2 concentration increase within 3 days after SAH indicating that the CSF PLA_2 and PGE_2 would play a role in the early brain injury rather than cerebral vasospasm after SAH. A decline in GCS within 2 days after SAH shows the presence of the early brain injury in this study's population.

The change in concentration of a chemical substance in CSF would reflect the change in its concentration in the extracellular space in the brain, although it takes time to diffuse from the extracellular space to CSF[12]. During injury, the BBB becomes leaky enough to pass large molecule including PLA_2. Prostaglandins are synthesized by brain neural tissue and blood vessels in brain[4], and eliminated rapidly by the uptake mechanism in the choroid

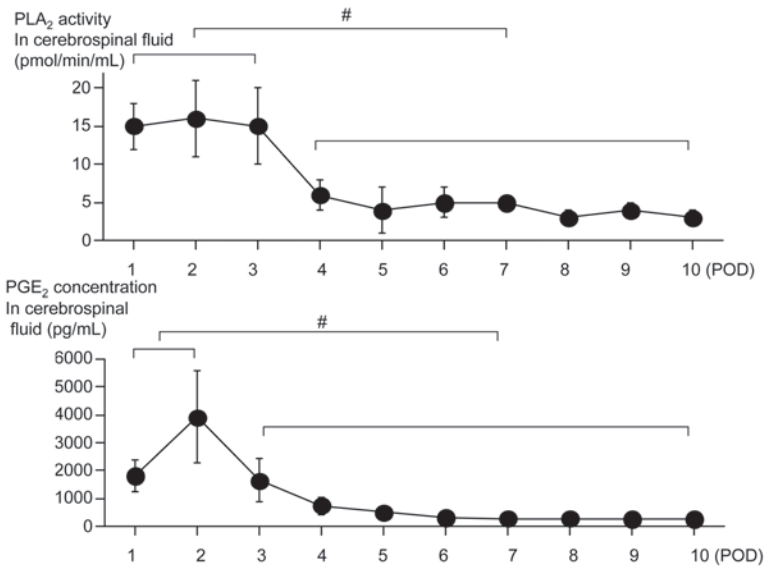

Fig. 2 The time course of phospholipase A_2 (PLA_2) activity (the upper) and prostaglandin E_2 (PGE_2) concentration (the lower) in cerebrospinal fluid after subarachnoid hemorrhage. Data are expressed as mean ± SEM. # $p<0.05$ compared with value of other day.

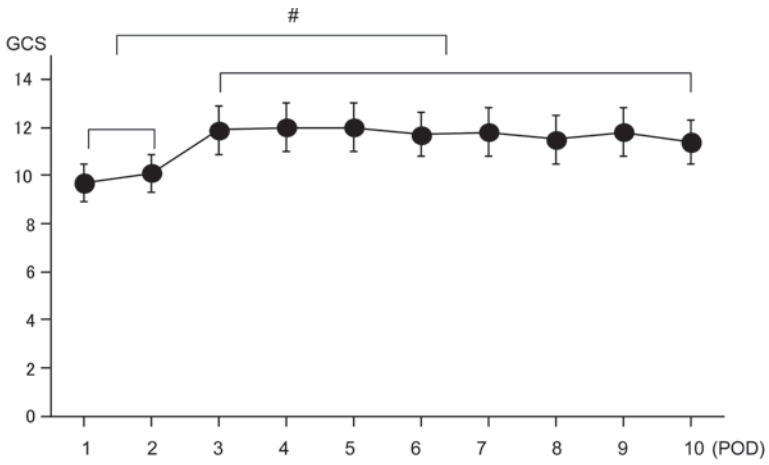

Fig. 3 The time course of Glasgow Coma Scale (GCS) score after subarachnoid hemorrhage. Data are expressed as mean ± SEM. # $p<0.05$ compared with value of other day.

plexus[13]. Pickard et al.[13] suggested that the disturbance of the CSF circulation after SAH might impair the clearance of eicosanoid. Thus, Increases in PLA_2 activity and PGE_2 concentration in CSF might reflect both increased production in the brain and decreased clearance from CSF.

The important result of the early brain injuries after SAH is the alteration of BBB permeability. Dysfunction of BBB contributes to brain edema and elevation in ICP after SAH. However, postoperative ICP monitoring systems with ventricular catheter are not routinely used for all postoperative SAH patients in our hospital because ventricular drainage increases the risk of aneurysm rebleeding[14]. Brain edema is difficult to evaluate on CT scans[15]. Because we could not analyze early brain injury quantitatively, we selected GCS as the index of neurologic function. Deteriorated GCS in early stage after SAH showed the presence of the early brain injury in this study's population.

It was reported that free fatty acids including arachidonic acid, substrate of eicosanoids, of CSF were initially elevated within 3 days and secondly elevated between 8 and 10 days after SAH[9]. Vinge et al.[8] demonstrated that an infusion of arachdonic acid into cerebral arteries produced widespread arteriolar occlusions, severe neurological deficits and endothelial cell damage. Pilitsis et al.[9] reported that free fatty acids may be involved in the cascade of deleterious events that follows SAH by causing the destruction of the electrochemical potential in mitochondria and resultant cellular edema. The present study indicates that the CSF PLA_2 activity increases within 3 days, and there is no second increase. Although the reason of this discrepancy is not clear, the possible explanation can be advanced as follows. Integrity of the blood brain barrier in the early stage of SAH might have improved not to leak the relatively large molecular weight substance including PLA_2 in the second period.

Shapira et al.[1] reported that closed head trauma increased brain tissue PGE_2 concentration, and that increase was associated with cerebral edema formation and worsening of the neurologic severity score. Although the number of patients was not sufficient and its measurements was not sequential, Pickard et al.[13] demonstrated that CSF eicosanoid level increased abruptly following SAH, and decreased with time in accordance with the findings of the present study. Walker et al.[16] pointed out that the cerebrospinal fluid level of PGE_2 was unreliable for the marker of brain circulation and too small amount of markers to induce delayed vasospasm. Increased CSF PGE_2 concentration after SAH may play a role in the early brain injury, rather than cerebral vasospasm.

In conclusion, the CSF PLA_2 activity and PGE_2 concentration increased within 3 days after SAH accompanied with decline of GCS. These substances may play a role in the acute brain injury rather than delayed cerebral vasospasm.

(Received: April 15, 2005)

This research was supported by research funds to promote the hospital functions of Japan Labour Health and Welfare Organization.

This work presented, in part, at the meeting of the Americal Society of Anesthesiologist, San Francisco, CA, October 11-15,2003

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（原稿受付　平成17. 4. 15）

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クモ膜下出血後，動脈瘤クリッピング術後の血漿及び髄液中のホスホリパーゼA2とプロスタグランジンE2濃度の変動

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—キーワード—
クモ膜下出血，ホスホリパーゼA_2，プロスタグランジンE_2

我々はアラキドン代謝の律速酵素であるホスホリパーゼA2（PLA2）と代謝産物であるプロスタグランジンE2（PGE2）の髄液と血漿中濃度の経時的変化について測定し，クモ膜下出血（SAH）後の脳血管攣縮や急性脳傷害との関連について検討した．対象は本研究の被検者として親族より同意が得られ，長崎労災病院においてSAH後に脳動脈瘤クリッピング術が行われ，脳槽ドレーンが10日以上挿入されていた患者10人である．破裂した脳動脈瘤はSAH発症後24時間以内に開頭クリッピング術が行われ，脳槽ドレーンが挿入された．脳脊髄液は間欠的にドレナージされ，頭蓋内圧が20mmHg以下になるように維持された．術翌日（1POD）から術後10日目（10POD）まで毎日午前中に髄液と血液を採取し，血漿中及び髄液中のPLA2及びPGE2を測定した．意識障害の指標としてグラスゴーコーマスケール（GCS）を同時に判定した．

PLA2とPGE2の血漿濃度は経時的変化については，有意差はなかった．PLA2とPGE2の髄液中濃度は，それぞれ1～3POD，1～2PODで4～10POD，3～10PODと比較して高値を示した．またGCSは1～2PODで3～10PODと比較して低下していた．

髄液中のPLA2とPGE2はクモ膜下出血後の急性期に増加しており，髄液中のアラキドン酸代謝産物はクモ膜下出血後の脳血管攣縮よりもむしろ急性脳傷害に関連していると示唆された．