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A non-enzymatic derived arachidonyl peroxide, 8-iso-prostaglandin $F_{2\alpha}$, in cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage participates in the pathogenesis of delayed cerebral vasospasm

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Abstract

We performed serial measurements of 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), a non-enzymatic derived arachidonyl peroxide, in the cerebrospinal fluid (CSF) of 34 patients with subarachnoid hemorrhage (SAH). Patients were treated with open or endovascular surgery within 48 h of onset. Delayed cerebral vasospasm was verified by the presence of a low-density area on CT scan indicating focal cerebral infarction occurring after symptomatic delayed vasospasm. Concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF of 15 patients exhibiting delayed cerebral vasospasm were compared with those of 19 patients who did not exhibit vasospasm. The concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF of patients showing vasospasm were 42.4 ± 37.1 pg/ml (mean $\pm$ S.D., $n = 12$) on Days 0–2, 66.4 ± 41.0 pg/ml ($n = 14$) on Days 3–5, 118.5 ± 89.9 pg/ml ($n = 15$) on Days 6–8, 86.2 ± 70.2 pg/ml ($n = 11$) on Days 9–11, 48.8 ± 31.8 pg/ml ($n = 10$) on Days 12–14, 27.8 ± 20.1 pg/ml ($n = 7$) after Day 20, while the concentrations in patients not showing vasospasm were 24.8 ± 12.0 pg/ml ($n = 18$) on Days 0–2, 25.7 ± 15.2 pg/ml ($n = 19$) on Days 3–5, 47.5 ± 52.3 pg/ml ($n = 18$) on Days 6–8, 56.7 ± 72.0 pg/ml ($n = 13$) on Days 9–11, 34.2 ± 53.1 pg/ml ($n = 15$) on Days 12–14, 20.1 ± 18.2 pg/ml ($n = 10$) after Day 20. CSF concentrations of 8-iso-$PGF_{2\alpha}$ on Days 3–5 and Days 6–8 were significantly higher in patients showing vasospasm as compared to patients not showing vasospasm. CSF levels of 8-iso-$PGF_{2\alpha}$ in patients showing vasospasm gradually increased in the days after onset of SAH and peaked on Days 6–8. Levels returned to normal after Day 20. These values on Days 3–5, Days 6–8, and Days 9–11 were significantly higher than the value after Day 20. Considering these data and the biological activities of 8-iso-$PGF_{2\alpha}$, such as development of inflammation, membrane perturbation and vasoconstriction, we conclude that 8-iso-$PGF_{2\alpha}$ may play a role in delayed cerebral vasospasm after SAH.

Keywords: 8-iso-Prostaglandin $F_{2\alpha}$; Lipid peroxides; Delayed cerebral vasospasm; Cerebrospinal fluid; Subarachnoid hemorrhage

Delayed cerebral vasospasm occurring after subarachnoid hemorrhage (SAH) is a serious problem because it affects the outcome of these patients [15]. Several reports have found evidence that free radicals and lipid peroxidation are involved in the pathophysiology of delayed cerebral vasospasm after SAH [13,19,22]. Lipid peroxidation initiated in the subarachnoid clot is candidate for mechanism of delayed cerebral vasospasm. It has been shown that levels of lipid peroxides in cerebrospinal fluid (CSF), detected as thiobarbituric acid-reactive substances, were significantly higher in SAH patients who manifested symptomatic vasospasm than in those who did not [18]. Leukotrienes were considered possible candidates for the spasmogenic substance because they were chemical mediators of inflammation, possessing potent chemotactic and vasoconstrictive properties [2,17,20,24]. Watanabe et al. [21] previously reported that 5-lipoxygenase activity was markedly increased in the

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basilar artery with vasospasm in a canine SAH model. They also showed that the subarachnoid clot contained a significant amount of 12-hydroxyeicosatetraenoic acid (12-HETE), a product of arachidonate 12-lipoxygenase. Sakamoto et al. [16] measured concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF, a basilar artery segment, and the subarachnoid clot using a canine SAH model. They found a significant increase in levels of 8-iso-$PGF_{2\alpha}$ in the CSF and in the basilar artery segment on Day 7 as compared with control animals. These results suggested that 8-iso-$PGF_{2\alpha}$ could play a role in the pathogenesis of the delayed cerebral vasospasm. Therefore, we measured 8-iso-$PGF_{2\alpha}$ concentrations in the CSF of patients with SAH.

The study included 34 patients with aneurysmal SAH (males: 12, females: 22, age range: 41–91 years, mean age 62.7 years) who were consecutively admitted to the Department of Neurosurgery, Tottori University Hospital, and three affiliated hospitals, from September 2001 to March 2004. The amount of subarachnoid clot were evaluated in CT scan by Fisher's method [1]. Four patients were in Fisher group 2, twenty-nine in group 3 and one in group 4. Thirty-three patients were treated with open surgery and one patient was treated with endovascular surgery, all within 48 h after the initial SAH attack. After surgery, silicon drainage tubes were placed in the CSF spaces for continuous drainage and CSF sampling. CSF samples were obtained mainly from the cisternal drainage, occasionally from ventricular or lumbar drainage at an interval of every few days. Between Day 6 and Day 18, 15 patients showed low-density areas on a computed tomography (CT) scan after exhibiting symptomatic delayed cerebral vasospasm. Nineteen patients did not showed any low-density areas due to vasospasm on CT scan. As a control we obtained CSF samples from 9 patients (males: 6, females: 3, age range: 33–75 years, mean age 60.1 years) with spondylosis. Samples were obtained at the time of myelography. CSF samples were centrifuged at 3,000 rpm for 5 min and the supernatants were stored at −80 °C until measurement of 8-iso-$PGF_{2\alpha}$. One milliliter of each sample was added to 800 μl of methanol and then homogenized for 1 min. Homogenates were then centrifuged at 10,000 rpm at 4 °C for 10 min. Pellets were discarded and the supernatants were subjected to further purification. All samples were subjected to alkaline hydrolysis and purified by Sep-Pak C18 columns (Waters Millipore, Milford, MA, USA). The 8-iso-$PGF_{2\alpha}$ content was measured as the sum of free and esterified 8-iso-$PGF_{2\alpha}$. Absorbance was monitored at 405 nm with a 96-well microplate reader (Multiscan MS-UV Labsystems, Farnborough, Hampshire, UK). The concentrations of 8-iso-$PGF_{2\alpha}$ in CSF are expressed as the mean ± S.D. (pg/ml) from the indicated number of samples. Statistical comparisons were made using the Mann–Whitney's *U*-test, and $p < 0.05$ was considered to be statistically significant.

As shown in Fig. 1, mean concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF of SAH patients showing vasospasm were 42.4 ± 37.1 pg/ml ($n = 12$) on Days 0–2, 66.4 ± 41.0 pg/ml

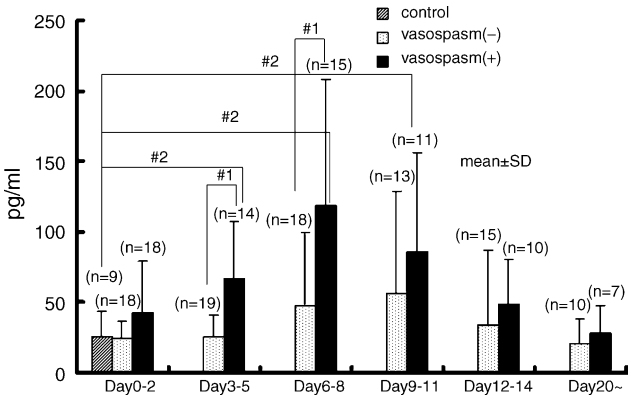

Fig. 1. Changes in CSF 8-iso-$PGF_{2\alpha}$ concentrations in SAH patients showing vasospasm and not showing vasospasm. CSF 8-iso-$PGF_{2\alpha}$ concentrations in patients showing vasospasm are significantly higher than those not showing vasospasm on Days 3–5 and Days 6–8 (Mann–Whitney's *U*-test, $^{\#1}p < 0.05$). CSF 8-iso-$PGF_{2\alpha}$ concentrations on Days 3–5, Days 6–8 and Days 9–11 in patients showing vasospasm are significantly higher than those in control group (Mann–Whitney's *U*-test, $^{\#2}p < 0.05$). Bars represent mean ± S.D. and *n* represent number of samples.

($n = 15$) on Days 3–5, 118.5 ± 89.9 pg/ml ($n = 15$) on Days 6–8, 86.2 ± 70.2 pg/ml ($n = 11$) on Days 9–11, 48.8 ± 31.8 pg/ml ($n = 10$) on Days 12–14, 27.8 ± 20.1 pg/ml ($n = 7$) after Day 20. The values on Days 3–5, Days 6–8, and Days 9–11 were significantly higher than the mean value from patients in the control group (25.9 ± 17.4 pg/ml, $n = 9$). They were also significantly different from values obtained after Day 20. Among SAH patients not showing vasospasm, mean concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF were 24.8 ± 12.0 pg/ml ($n = 18$) on Days 0–2, 25.7 ± 15.2 pg/ml ($n = 19$) on Days 3–5, 45.5 ± 51.6 pg/ml ($n = 19$) on Days 6–8, 51.1 ± 68.2 pg/ml ($n = 15$) on Days 9–11, 33.0 ± 50.0 pg/ml ($n = 17$) on Days 12–14, 20.0 ± 17.3 pg/ml ($n = 11$) after Day 20. The concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF of SAH patients showing vasospasm were significantly higher on Days 3–5 and Days 6–8 as compared to SAH patients not showing vasospasm. Fig. 2 showed the differences of the concentrations of 8-iso-$PGF_{2\alpha}$ between in Fisher group 2 and group 3. Among SAH patients in Fisher group 2, mean concentrations of 8-iso-$PGF_{2\alpha}$ in the CSF were 31.6 ± 11.3 pg/ml ($n = 4$) on Days 0–2, 23.5 ± 17.2 pg/ml

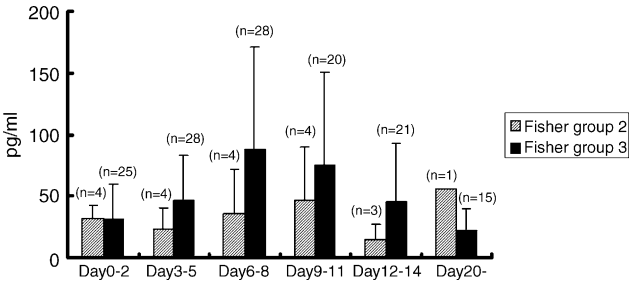

Fig. 2. Concentrations of 8-iso-$PGF_{2\alpha}$ in Fisher group 2 and Fisher group 3. The 8-iso-$PGF_{2\alpha}$ in CSF simply reflect amount of clot in CSF which shows much more in Fisher group 3.