Prostaglandins and Vasoactive Amines in Cerebral Vasospasm After Aneurysmal Subarachnoid Hemorrhage

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Five consecutively admitted patients with aneurysmal subarachnoid hemorrhage were treated with an indwelling lumbar spinal catheter. Daily samples of cerebrospinal fluid were analyzed for erythrocyte, protein, glucose, dopamine, epinephrine, serotonin, 5-hydroxyindoleacetic acid, tryptophan, histamine, thromboxane, 6-ketoprostaglandin F$_{10}$, prostaglandin E, and prostaglandin F$_{20}$ concentrations. The patients' neurologic grade on admission, hospital course, presence of vasospasm, level of consciousness, computed tomographic and angiographic findings, and outcome were compared with the concentrations of the above substances in the cerebrospinal fluid. All patients had elevated concentrations of serotonin, with the highest levels found early in the hospital course of the patients who developed vasospasm. Tryptophan content increased markedly in association with clinical and angiographic vasospasm. Concentrations of prostaglandin F$_{20}$ correlated highly with development of and fluctuations in clinical vasospasm, with angiographic findings, with neurologic grade on admission, and with outcome. Our results suggest that prostaglandin F$_{20}$ may be involved in delayed clinical vasospasm in patients with subarachnoid hemorrhage. (Stroke 1989;20:217-224)

Delayed onset of cerebral vasospasm after subarachnoid hemorrhage (SAH) continues to be a significant source of complications and poor outcome in the clinical management of patients with ruptured intracranial aneurysm. Although up to 70% of patients with aneurysmal SAH will demonstrate arteriographic narrowing in the major cerebral arteries, the reported frequency of clinically significant vasospasm is 20-40% in aneurysm patients, with anecdotal reports of vasospasm associated with other conditions. A number of risk factors for clinically significant vasospasm (such as the patient's neurologic grade on presentation, the amount of blood present in the basal cisterns on computed tomograms [CTs], a history of hypertension, age, and sex) and impaired cerebral vasoreactivity have been identified. However, the factors playing an etiologic role in the induction of cerebral vasospasm remain elusive.

The presence in the cerebrospinal fluid (CSF) of various substances such as blood and its breakdown by-products, oxyhemoglobin, vasoactive amines, serotonin, free radicals and oxidative agents, and more recently prostaglandins (PGs) have been implicated as potential causative factors.

The purpose of our study was to determine simultaneously the concentrations of selected vasoactive amines and PGs in the lumbar CSF of patients with SAH on successive days following the hemorrhage and to correlate clinical and radiographic findings with the development of vasospasm and the patients' outcomes.

Subjects and Methods

Our study was performed in compliance with Human Subject Review Committee guidelines. Five consecutively admitted patients with a diagnosis of aneurysmal SAH were treated by placement of an indwelling subarachnoid spinal catheter for daily (morning) collection of CSF for laboratory analysis. When indicated, a ventricular catheter was placed for monitoring of intracranial pressure (ICP). The patients' neurologic grades, levels of consciousness (LOC), sensory and motor functions, clinical signs of vasospasm, angiographic findings, and treatment were detailed in the medical records. Patients were
evaluated each morning before the collection of CSF, and if there was evidence of noncommunicating hydrocephalus or an intracranial mass lesion, the sample was not collected pending further diagnostic evaluation. No patient suffered any complication related to the lumbar drain.

CSF (35 ml) was collected between 8:00 and 10:00 AM into a 50-ml centrifuge tube containing 10 mg indomethacin and 0.7 ml of 5 mM neutralized reduced glutathione on ice. Samples were centrifuged at 1200g for 20 minutes in a refrigerated centrifuge at 4° C. The supernatant was carefully aspirated and placed on ice.

Five milliliters of the supernatant was used to assay either thromboxane B₂ (TxB₂), 6-ketoprostaglandin F₁α (6-kPGF₁α), PGF₂α, or PGE; CSF was acidified with 6N HCl (0.1 ml/1.0 ml CSF) and spiked with 2000 cpm of [³H]TxB₂, [³H]6-kPGF₁α, [³H]PGF₂α, or [³H]PGE for recovery purposes. All samples were stored in a freezer at -20° C. Acidified CSF was thawed at room temperature; the samples were extracted three times with chloroform (40 ml total volume), the chloroform was evaporated under a stream of nitrogen, and the dried samples were then frozen at -20° C until the assay. TxB₂ (the stable metabolite of Txₐ), 6-kPGF₁α, PGF₂α, and PGE in the dried extracts were quantified by radioimmunoassay as described earlier.³⁴,³⁵ The minimum detectable amount in 1 ml CSF was 20 pg/ml for TxB₂ and 6-kPGF₁α and 25 pg/ml for PGF₂α and PGE.

For assays of catecholamines and serotonin, 2.5 ml CSF was acidified with an equal volume of 0.4N perchloric acid. All samples were stored in a freezer at -20° C. Catecholamines, serotonin and its metabolites, and tryptophan in the acidified CSF supernatant were quantified by reverse-phase high-performance liquid chromatography with electrochemical detection as described by Krstulovic³⁶ and Holly and Makin.³⁷ Internal standards were prepared and chromatographed. The ratio of the internal standard (3,4-dihydroxybenzylamine, DHBA) peak height in the sample to that in a mixture of the standard gives a recovery value for each sample. Each value was corrected for recovery.

For the analysis of histamine, 5 ml of 0.4 M perchloric acid was added to 5 ml of the supernatant resulting from centrifugation of the CSF. After centrifugation of this mixture, duplicate aliquots of the supernatant were used to quantify the histamine content by the fluorometric method of Shore et al³⁸ (except that 1.5N phosphoric acid was used to stabilize the fluorescent product).

**Case Reports**

Patient 1 was a 47-year-old normotensive woman with a 1-week history of persistent headaches and a
sudden collapse on the evening of admission (Figure 1). Patient 1 had a markedly stiff neck, was hypertensive to 200/105 torr, would open her eyes to stimulation but would not speak or follow commands, and had bilaterally upgoing toes without any motor weakness. She was judged to be Grade IV on the Hunt and Hess Scale. CT demonstrated intraventricular hemorrhage and noncommunicating hydrocephalus in addition to diffuse subarachnoid blood in the basal cisterns, both Sylvian fissures, and the interhemispheric fissure. A ventriculostomy was placed, and ICP was normalized. Angiography revealed a left anterior communicating artery aneurysm. A lumbar drain was placed. Her LOC improved for the next 2 days. On the afternoon of Day 3 after SAH, Patient 1 demonstrated transient right arm weakness. Her symptoms progressed, with decreasing LOC despite volume treatment for vasospasm, and by Day 4 she was obtunded and had a fixed right hemiplegia. On Day 6 she had a further decrease in her LOC, suffered a seizure, and was placed on a ventilator. She remained comatose with extensor posturing, and angiography documented severe vasospasm. She developed fixed pupils on Day 8. A radionuclide scan revealed no cerebral blood flow, treatment was terminated, and she died.

Patient 2 was a 50-year-old normotensive man who suffered an ictus on the morning of admission (Figure 1). He was sleepy and confused, with a mild expressive aphasia. He was judged as being Grade III. CT demonstrated SAH and layering of clot in the basal cisterns. Angiography revealed a large left internal carotid artery aneurysm. A lumbar drain was placed. Patient 2 had an improving LOC until Day 4 after SAH, when he was noted to become lethargic again and was treated with volume expansion. On Day 5, he was sleepy and had a right hemiparesis and dysphasia. He had a fluctuating course with resolution of his hemiparesis over the next 3 days. On Day 9, the internal carotid artery aneurysm was clipped. The next day he developed fluctuating hemiplegia and aphasia and progressed to develop fixed deficits despite vigorous therapy. Angiography revealed cerebral vasospasm. In the next 2 months he had good recovery from dysphasia, had moderate spastic monoplegia, and required shunting for hydrocephalus.

Patient 3 was a 38-year-old hypertensive black man admitted 2 hours after the sudden onset of headache and collapse associated with vomiting and transient loss of consciousness (Figure 1). He was given a neurologic grade of III as he was sleepy and confused but without weakness. CT revealed diffuse blood in the basal and ambient cisterns and Sylvian fissures and a small clot in the fourth ventricle without hydrocephalus. By Day 2 after SAH he had a stiff neck and an intact LOC. Angiography demonstrated two aneurysms, one at the right internal carotid–posterior communicating artery junction and one at the right internal carotid artery bifurcation. A lumbar drain was placed. On Day 9, Patient 3 underwent clipping of his aneurysms. He had a benign postoperative course, was neurologically intact at discharge, and had returned to his job within a month.

Patient 4 was a 61-year-old normotensive woman with a sudden severe headache associated with vomiting but without loss of consciousness (Figure 1). She was admitted on Day 3 after her SAH with the complaint of double vision (Figure 1). CT revealed a left anterior communicating artery aneurysm. Angiography revealed three aneurysms, one at the right internal carotid–posterior communicating artery junction, one at the basilar artery bifurcation, and another at the left middle cerebral artery bifurcation. A lumbar drain was placed. On Day 11 after her SAH she underwent clipping of the ruptured internal carotid artery aneurysm and the incidental basilar artery aneurysm. She had a benign course and was discharged with an intact neurologic status.

Patient 5 was a 50-year-old right-handed hypertensive woman admitted on Day 0, the day of her SAH. She was judged to be Grade III as she was lethargic and confused without a focal neurologic deficit (Figure 1). CT revealed hydrocephalus and diffuse SAH with layering of clot in the basal cisterns and the occipital horns of the lateral ventricles. A ventriculostomy was performed and a lumbar drain was placed. Angiography demonstrated a right anterior communicating artery aneurysm. She had a progressive improvement in her LOC and mentation through Day 5. On Day 6 she became confused and was treated vigorously with fluids. The next day she became more lethargic and developed hemiparesis. She had progressive deterioration of her neurologic status. CT revealed no evidence of rebleeding; angiography revealed severe vasospasm. She required intubation by Day 9 and died early on Day 11.

CSF was also collected from nine controls (three healthy volunteers, three patients with incidental aneurysms, and three asymptomatic patients 9 months after treatment for SAH). Analysis of these samples yielded similar values, and they were pooled to establish baseline concentrations.

Results

There were large variations in the CSF erythrocyte counts among patients and in the same patient over time. There was no correlation between the initial erythrocyte count and neurologic grade. Patients 1, 2, and 5 (who developed vasospasm) had erythrocyte counts on Day 1 of >400,000/ml. However, our sample is too small to evaluate for significance. By the end of the first week there was a 90% drop in the erythrocyte concentration in the lumbar CSF (Figure 2).

Although CSF protein concentration tended to be elevated early after SAH, it quickly returned to
baseline within the first 24–48 hours. Glucose concentrations remained within normal limits. The mean±SEM baseline concentration of dopamine in the CSF was 280±100 ng/ml. In SAH patients, dopamine concentration tended to be elevated and ranged from undetectable levels to concentrations as high as 2200 ng/ml. There was no tendency for dopamine concentration to change with vasospasm; the levels drifted toward baseline and were within the normal range by Day 11. Baseline concentrations of epinephrine in the CSF were <100 ng/ml. Except for one patient with narcotic addiction, CSF concentrations in the SAH patients remained within normal limits. There was no correlation between epinephrine concentration and neurologic grade on admission, vasospasm, or outcome.

Mean±SEM baseline concentration of serotonin in the CSF was 60±9.7 ng/ml. SAH patients had significantly (p<0.001) elevated concentrations, with daily means generally above 200 ng/ml (Figure 3). Although Patients 1, 2, and 5 attained serotonin concentrations well above 250 ng/ml at some time during their hospital course, there was no correlation between serotonin concentration and neurologic grade on admission, vasospasm, or outcome.

Controls had undetectable amounts of 5-hydroxy-indoleacetic acid (5-HIAA) even though our assay was sensitive to concentrations as low as 25 ng/ml. SAH patients tended to have elevated 5-HIAA concentrations, ranging from undetectable to 150 ng/ml. No correlation was found between the CSF content of 5-HIAA and neurologic grade, vasospasm, or outcome. Patients 1, 2, and 5 had elevated concentrations of histamine (above the baseline value of 15 ng/ml) at some time during their hospital course. However, daily histamine concentrations did not correlate with the neurologic grade on admission or with clinical or radiologic vasospasm.

Mean±SEM baseline concentration of tryptophan in the CSF was 1400±657 ng/ml; all SAH patients had normal concentrations for the first 2 days. Development of clinical vasospasm in Patients 1, 2, and 5 was preceded by significant (p<0.01) elevation and recovery from vasospasm was associated with a drop in tryptophan concentration (Figure 4). Patients 1 and 5 (who died of vasospasm) had increasing tryptophan concentrations (to >2000 ng/ml) that persisted until death. Surgery was followed by small increases in tryptophan content.

Mean±SEM baseline concentration of TxB₂ in the CSF was 440±32 pg/ml; SAH patients had concentrations that fluctuated from one half to twice that. There was no relation between TxB₂ concentration and neurologic grade on admission, vasospasm, or outcome (Figure 5).

Mean±SEM baseline concentration of 6-kPGF₁α was 1400±100 pg/ml; the concentration varied widely among SAH patients (Figure 6). There was no correlation between 6-kPGF₁α concentration or the 6-kPGF₁α:TxB₂ ratio and neurologic grade on admission, vasospasm, surgery, or outcome.

Mean±SEM baseline concentration of PGE in the CSF was 450±50 pg/ml. All SAH patients except one had depressed concentrations of PGE compared with controls (Figure 7). CSF concentra-
Figure 4. Changes in tryptophan concentration in lumbar cerebrospinal fluid (CSF) after subarachnoid hemorrhage (SAH). Numerals 1 through 5 represent values from corresponding patients. SAH occurred on Day 0. Control, baseline concentrations from three healthy volunteers, three patients with incidental aneurysms, and three asymptomatic SAH patients 9 months after surgery. S, surgery; D, death secondary to vasospasm. V, onset of right hemiplegia and aphasia due to vasospasm.

Mean±SEM baseline concentration of PGF_{2\alpha} in the CSF was 430±19 pg/ml. Patients 3 and 4 had low concentrations; only the three patients who developed vasospasm had elevated concentrations (Figure 8). PGF_{2\alpha} concentrations were significantly \((p<0.03)\) elevated in the presence of clinical vasospasm. Fluctuations in LOC attributed to vasospasm correlated significantly \((p<0.03)\) with the daily changes in PGF_{2\alpha} concentration as measured for directional changes using the \(\chi^2\) test. Patients 1 and 5 had PGF_{2\alpha} concentrations well above 1000 pg/ml. PGF_{2\alpha} concentrations were elevated in the presence of radiologic arteriospasm.

Discussion

A total of 37 serially collected CSF samples from five patients with SAH were submitted to laboratory analysis. The process of erythrocyte lysis in the CSF proceeded rapidly, with a >90% drop in the cell count by the end of the first week. Accumulation of erythrocyte breakdown by-products may therefore coincide with the statistical peak of cerebral vasospasm, approximately 1 week after SAH. An initial erythrocyte count of >400,000/ml in the lumbar CSF may herald vasospasm, with...
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dent aneurysms, and three asymptomatic SAH patients
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vasospasm; V, onset of right hemiplegia and aphasia due
to vasospasm. Arrows show direction of changes in level
of consciousness associated with fluctuations in severity
tion at the onset of clinical vasospasm may
ted in the pathogenesis of vasospasm after mock
SAH21 although treatment with PGI has failed to
improve delayed vasospasm. 22-23 Rodriguez et al31
found decreased 6-kPGF ia concentrations in the
lumbar CSF of a patient with SAH who developed
vasospasm; other patients with SAH had elevated
levels of PGI.32 Our study confirms that PGI, mea-
sured as 6-kPGF, a, is the major PG in the CSF, with
a mean±SEM baseline concentration of 1400±100
pg/ml. Serial sampling of CSF produced a wide
range of 6-kPGF ia concentrations without correla-
tion to clinical status or outcome (Figure 6). Fur-
depress collateral blood flow after induced isch-
emia40,41 and has been implicated in cerebral arterial
spasm.42,43 Serotonin may well play a role in the
reported decrease of cerebral blood flow after aneu-
rysmal SAH.44,45 Although serotonin concentrations
were high in all our SAH patients and still
higher in those patients who developed vasospasm,
serotonin concentration did not correlate with clin-
ical fluctuations due to vasospasm. It appears that,
if serotonin participates in the development of vaso-
spasm, it is more likely to be responsible for "setting
of the vascular tone" or enhancing vascular reac-
tivity to yet another agent that more directly induces
clinical vasospasm.

CSF concentrations of 5-HIAA, dopamine, epi-
nephrine, and histamine were elevated but did not
correlate with the patients' neurologic grade on
admission, development of clinical or angiographic
vasospasm, surgical procedure, or outcome. It is
unlikely that these substances are involved in
vasospasm.

PGs are the products of cyclooxygenation of
arachidonic acid, which is released from membrane
phospholipids. Brain tissue and blood vessels syn-
thesize these eicosanoids and are rich stores of
these vasoactive substances.27,29 Over the past de-
cade there has been an increasing body of evidence
pointing toward the role of PGs in the normal
process of cerebral autoregulation as well as patho-
logic processes such as edema formation and develop-
ment of delayed clinical vasospasm after aneu-
rysmal SAH.16,21,22,27,46,47 Increased concentrations
of TxB2, 6-kPGF ia, and PGF 2a in the CSF have been
noted after induced SAH.48

TxB2 is the stable by-product of TxA2, which has
strong vasoconstrictive and platelet aggregating prop-
erties. Komatsu et al26 demonstrated alleviation of
vasospasm in dogs treated with TxA2 synthetase
inhibitor. Seifert et al32 compared the clinical course
of four patients with their CSF concentrations of
6-kPGF ia and TxB2 and suggested that TxB2 may
serve as an indicator for the risk of developing
cerebral vasospasm. Our serial measurements of
TxB2, as demonstrated in Figure 5, did not reveal
any correlation to clinical vasospasm or outcome.
The stable metabolite of PGI, 6-kPGF ia is a pros-
tanoid that promotes vasodilation and antiaggrega-
tion and increases blood flow. PGI inhibits TxA2
and angiotensin II.25 PGI deficiency has been impli-
cated in the pathogenesis of vasospasm after SAH21
although treatment with PGI has failed to
improve delayed vasospasm.22,23 Rodriguez et al31
found decreased 6-kPGF ia concentrations in the
lumbar CSF of a patient with SAH who developed
vasospasm; other patients with SAH had elevated
levels of PGI.32 Our study confirms that PGI, mea-
ured as 6-kPGF ia, is the major PG in the CSF, with
a mean±SEM baseline concentration of 1400±100
pg/ml. Serial sampling of CSF produced a wide
range of 6-kPGF ia concentrations without correla-
tion to clinical status or outcome (Figure 6). Fur-

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FIGURE 8. Changes in prostaglandin F 2a (PGF2a)
concentration in lumbar cerebrospinal fluid (CSF) after sub-
arachnoid hemorrhage (SAH). Numerals 1 through 5
represent values from corresponding patients. SAH
occurred on Day 0. Control, baseline concentrations
of clinical vasospasm. Neurologic grade on admission is
indicated by Roman numeral in parentheses.

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poor outcome. The increase in CSF protein concentra-
tion after SAH, however, was quite transient and
suggests an active process for clearing excess pro-
tein and maintaining CSF homeostasis. A dysfunc-
tion of this active clearing process after SAH may
result in the accumulation of substances that pro-
mote cerebral vasospasm.

The observed increase in CSF tryptophan concen-
tration at the onset of clinical vasospasm may
indicate dysfunction of CSF homeostasis. Trypt-
ophan content was initially normal and did not
correlate with neurologic grade on admission; how-
ever, later fluctuations of tryptophan concentration
preceded the development of and recovery from
clinical vasospasm (Figure 4). Tryptophan is pro-
duced by a number of metabolic pathways, and its
concentration in the CSF appears to be predictive of
clinical vasospasm even though tryptophan is not
known to have significant vasoactive properties.

Serotonin is a potent vasoactive substance; it is
present in the cerebrovascular sympathetic nerves,39
produces marked vasoconstriction when applied
topically,20 is abundant in platelets, and is released
from blood clots.14 Serotonin has been shown to
but the 6-kPGF<sub>2α</sub>:TxB<sub>2</sub> ratio was not of any predictive value. It is unlikely that either of these prostanoids plays a critical role in the induction of clinical vasospasm.

Intracarotid injection of PGE<sub>1</sub> reportedly alleviates vasospasm and increases blood flow after induced SAH in baboons. In our study, however, the concentration of total PGE in CSF or its fluctuations failed to correlate with neurologic grade on admission or clinical vasospasm. We have no evidence that PGE plays a role in cerebral vasospasm.

PGF<sub>2α</sub> is an extremely potent and fast-acting vasoconstrictor. Physiologic concentrations of PGF<sub>2α</sub> produce rapid contractions in isolated human pial arteries and canine basilar arteries that are approximately twice as strong as those induced by fresh whole blood. An increased PGF<sub>2α</sub> concentration in the CSF has been reported after induced SAH. It is noteworthy that in studies of transient brain ischemia, restoration of blood flow is followed by an increase in PGF<sub>2α</sub> content, which reaches a peak at 2 hours and then subsides, paralleling the course of postischemic cerebral hyperperfusion. Clinical studies have reported significantly increased concentrations of PGF<sub>2α</sub> in the lumbar CSF shortly after SAH and approximately 1 week later.

In our study only Patient 1 (who presented with a poor neurologic grade, Grade IV) had a markedly elevated PGF<sub>2α</sub> concentration in the CSF on admission; subsequent levels were elevated only in patients with clinical vasospasm. The severity of vasospasm correlated significantly (p<0.03) with the CSF concentration of PGF<sub>2α</sub>. Values of >1000 pg/ml preceded death due to untreatable vasospasm, and moderate elevations above baseline were associated with clinical vasospasm (Figure 2). It appears that PGF<sub>2α</sub>, the action of which is blocked by the calcium channel blocker nimodipine, is of value for predicting clinical vasospasm following SAH. Increased CSF concentration of tryptophan after SAH may indicate disruption of the active CSF homeostatic mechanism, allowing for accumulation of PGF<sub>2α</sub>, which, in the presence of a sustained elevation of serotonin concentration, may participate in the induction of delayed cerebral vasospasm. Further study of the roles of tryptophan, serotonin, and PGF<sub>2α</sub> in cerebral vasospasm is underway.

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