CSF smooth-muscle constrictor activity associated with cerebral vasospasm and mortality in SAH patients


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Cerebrospinal fluid (CSF) was collected preoperatively (by lumbar puncture) or perioperatively (by lumbar or ventricular drain) from 32 patients with subarachnoid hemorrhage (SAH) from ruptured intracranial aneurysms. Samples were also obtained from six control patients without evidence of subarachnoid blood. Smooth-muscle constrictor activity in the CSF was measured by bioassay using the isolated rat stomach fundus preparation. Concentrations of unidentified smooth-muscle constrictor substances were considerably greater in CSF from a group of seven patients with evidence of severe angiographic vasospasm and/or delayed ischemic deficits who died (73.8 ± 39.7 nmol/liter prostaglandin E2 (PGE2) equivalents), as compared to 25 other SAH patients who survived (6.5 ± 1.4 nmol/liter PGE2 equivalents), and six control patients (1.17 ± 0.34 nmol/liter PGE2 equivalents). The data suggest that there is a relationship between smooth-muscle constrictor substances in the CSF after SAH and both the degree of angiographic vasospasm and the outcome. It is possible that this relationship might be exploited clinically.

KEY WORDS · cerebral vasospasm · subarachnoid hemorrhage · cerebral aneurysm · smooth-muscle constrictor · cerebrospinal fluid · bioassay

SUBARACHNOID hemorrhage (SAH) from rupture of intracranial arterial aneurysms may be followed by cerebral vasospasm. The concomitant cerebral ischemia and neurological deficits are associated with increased morbidity and mortality. The capricious nature of pathological cerebral arterial spasm has made investigation of this phenomenon difficult, and its etiology remains obscure. However, in 1964, Buckell demonstrated the presence of vasoconstrictor material in cerebrospinal fluid (CSF) and hematoma fluid from SAH patients, implying that the syndrome had a pharmacological basis. Subsequently, Blaso and Boullin reported a study of the vasoconstrictor activity in CSF collected from patients after aneurysm surgery. Increasing CSF vasoactivity was associated with angiographic cerebral vasospasm leading to morbidity and mortality. Attempts to extend these observations to the period when pathological vasospasm has most often been reported (4 to 17 days post-SAH) by sampling CSF before conventionally timed surgery and after early surgery failed to reveal any close relationship between vasoconstrictor activity and morbidity.

We are reporting a study of 32 patients with SAH, showing that high levels of smooth-muscle constrictor activity in the CSF are associated (although not invariably) with severe angiographic vasospasm, delayed ischemic deficits, and subsequent mortality.

Clinical Material and Methods

Collection of CSF

Cerebrospinal fluid was collected by lumbar puncture after the patients' admission, and at operation by lumbar or ventricular drain, from 32 patients with SAH. We also obtained CSF from six control patients (Table 1) without evidence of subarachnoid blood. Overly red-colored CSF was centrifuged for 5 minutes at 6500 G to remove whole cells and particulate matter. The CSF was then frozen to −20°C until required for bioassay. In a single patient (Case 2, Table 2), serial CSF samples were obtained by repeated lumbar puncture. In three patients (Cases 3, 6, and 17, Table 2), both lumbar and ventricular CSF samples were collected.

Bioassay for Vasoconstrictor Activity

Male Sprague-Dawley rats were stunned, then sacri-
TABLE 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Vasoactivity (nmol/liter PGE2)</th>
<th>No. of Bioassays</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>dementia</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td>34</td>
<td>dementia</td>
<td>0.8</td>
<td>4</td>
</tr>
<tr>
<td>35</td>
<td>hydrocephalus</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>36</td>
<td>acoustic tumor</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td>37</td>
<td>dementia</td>
<td>1.7</td>
<td>7</td>
</tr>
<tr>
<td>38</td>
<td>CSF rhinorrhea</td>
<td>2.4</td>
<td>1</td>
</tr>
</tbody>
</table>

Mean ± SE 1.17 ± 0.34

* Control patients had no evidence of subarachnoid blood. Vasoconstrictor activity was measured on the isolated rat fundus. Values are given in prostaglandin E2 (PGE2) equivalents (nmol/liter) for 500 μl of cerebrospinal fluid (CSF) added to a 5-ml bath.

A. H. Kaye, et al.

TABLE 2

<table>
<thead>
<tr>
<th>CSF Samples</th>
<th>SMC Activity (nmol/liter PGE2)</th>
<th>No. of Bioassays</th>
<th>Spasm</th>
<th>Day Post-SAH</th>
<th>Admision</th>
<th>Mid-Term</th>
<th>Day Post-SAH</th>
<th>Surgery &amp; Day Post-SAH</th>
<th>Outcome (at 3 mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 lumbar</td>
<td>61.0</td>
<td>1</td>
<td>severe</td>
<td>6</td>
<td>III------V</td>
<td>6------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>2 lumbar</td>
<td>302.4</td>
<td>1</td>
<td>severe</td>
<td>7</td>
<td>III------V</td>
<td>18------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>3 ventric</td>
<td>74.0</td>
<td>2</td>
<td>not done</td>
<td>10</td>
<td>III------V</td>
<td>17------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>4 lumbar</td>
<td>31.5</td>
<td>2</td>
<td>not done</td>
<td>6</td>
<td>II-------</td>
<td>V-------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>5 lumbar</td>
<td>35.1</td>
<td>1</td>
<td>not done</td>
<td>7</td>
<td>II-------</td>
<td>V-------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>6 ventric</td>
<td>2.6</td>
<td>2</td>
<td>not done</td>
<td>6</td>
<td>IV-------</td>
<td>V-------</td>
<td>no surgery</td>
<td>died</td>
<td></td>
</tr>
<tr>
<td>7 lumbar</td>
<td>0.0</td>
<td>1</td>
<td>not done</td>
<td>13</td>
<td>I-------III</td>
<td>4---9</td>
<td>16</td>
<td>fair</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SE 73.8 ± 39.7

8 lumbar

9 lumbar

10 lumbar

Mean ± SE 6.7 ± 2.9

11 lumbar

12 lumbar

13 ventric

Mean ± SE 4.2 ± 1.0

14 lumbar

15 lumbar

16 ventric

17 ventric

18 lumbar

19 lumbar

20 lumbar

21 lumbar

22 lumbar

23 lumbar

24 ventric

25 lumbar

26 lumbar

27 lumbar

28 lumbar

29 lumbar

30 lumbar

31 lumbar

32 lumbar

Mean ± SE 6.8 ± 1.8

* Cerebrospinal fluid (CSF) samples were obtained from the lumbar or ventricular subarachnoid space. The day of SAH was designated Day 0. Concentrations of smooth-muscle constrictor material (SMC Activity) were estimated on the isolated rat fundus. Values for SMC activity in CSF are given in PGE2 equivalents (nmol/liter) for 500 μl added to a 5-ml bath. Spasm denotes the degree of arterial narrowing seen at angiography (see the Methods section for definitions). The clinical condition of patients was estimated on the scale described by Hunt and Hess.21 Grading was performed after admission, at surgery (if performed), and, in some cases, where there was maximal improvement or deterioration in the intervening period (Mid-Term). Outcome is defined in the Methods section.

J. Neurosurg. / Volume 60 / May, 1984
Data were analyzed by linear regression and an unpaired two-tailed t-test.

Patients who died were ranked 4. A good result meant that the patient could not resume his original occupation due to residual neurological deficits, although all these patients showed signs of delayed ischemic deficits, with associated cerebral ischemia and a subsequent poor outcome.

The clinical condition of the patients was rated according to the scale of Hunt and Hess. Patients were graded on admission, at the time of CSF sampling, and at the time of surgery. Patients underwent additional grading at times of maximal clinical improvement or deterioration following admission.

Outcome

Outcome of patients admitted with SAH was assessed 3 months after admission. Patients able to return to their former life-style and occupation were regarded as having a good outcome. A fair result meant that the patient could not resume his original occupation due to residual neurological deficits, although all these patients were able to return to a modified or part-time occupation. The outcome in patients who were unable to work at 3 months after admission and needed assistance was described as “poor.” For purposes of data analysis, a good outcome was ranked 1, fair was 2, and poor was ranked 3. Patients who died were ranked 4.

Samples of Cerebrospinal Fluid

The CSF was sampled from six control patients (Table 1) and 32 patients admitted with SAH after aneurysm rupture (Table 2). The CSF was collected between 3 and 42 days after SAH, with a mean time and standard error of collection at 11.1 ± 1.5 days. In a single patient (Case 2), four samples of CSF were obtained at intervals of between 7 and 14 days after SAH. The values for the last collected samples only are presented in Table 2. In three patients (Cases 3, 6, and 17), both lumbar and ventricular CSF samples were obtained. Times of sampling for these patients are given in Table 3.

Angiographic and Clinical Assessment of Vasospasm

Cerebral angiography was performed in 26 patients between 6 and 29 days after the initial hemorrhage (mean 10.9 ± 1.1 days). Severe angiographic vasospasm was detected in seven (27%) patients (Cases 1, 2, 3, 11, 14, 15, and 16). In four patients (Cases 1, 2, 3, and 11), this severe angiographic vasospasm was accompanied by delayed ischemic deficits, resulting in death in Cases 1, 2, and 3. A single patient (Case 10) had angiographic spasm of moderate severity, with associated cerebral ischemia and a subsequent poor outcome.

Mild cerebral vasospasm was demonstrated at angiography in four patients (Cases 17 to 20), with accompanying neurological deficits in one (Case 17). Six patients (Cases 4 to 9) had severe neurological deficits (Grade IV) at the time of CSF sampling, but angiography was not performed due to their poor condition.

Outcome

Of 32 patients with SAH, seven (21.9%) died before surgery could be performed, between 6 and 18 days after SAH (mean 10.4 ± 2.1 days, Table 2). All these patients showed signs of delayed ischemic deficits, with angiographic confirmation of vasospasm in three (Cases 1, 2, and 3).

Three patients (9.5%) had a poor outcome. They were in Hunt and Hess Grade III and IV on admission (Cases 8, 9, and 10). Only one of these (Case 10) had angiography, and subsequent aneurysm surgery 30 days after SAH.

A fair outcome was seen in three patients (9.5%) all of whom underwent aneurysm surgery. Of these patients, Case 11 showed severe angiographic vasospasm with concomitant neurological deficits 7 days after SAH, but recovered sufficiently for successful aneurysm surgery to be performed on Day 38.

A good outcome resulted in 19 patients (59%), all of whom underwent aneurysm surgery. In this group, a single patient (Case 17) developed delayed neurological deficits on Day 12. However, these had resolved sufficiently by Day 29 to allow angiography (which demonstrated mild vasospasm), and surgery on Day 30, following which there was a good recovery.
**Smooth-Muscle Constrictor Activity in CSF**

**Lumbar Versus Ventricular CSF.** Of 36 samples of CSF collected from 32 SAH patients (Table 2), 30 were obtained from the lumbar subarachnoid space, and six from the ventricles. The mean smooth-muscle constrictor activity in lumbar CSF samples was 20.1 ± 10.1 nmol/liter PGE\(_2\) equivalents. This was slightly, but not significantly (unpaired t-test), higher than the mean value detected in the ventricular CSF (14.6 ± 11.9 nmol/liter PGE\(_2\) equivalents). Too few samples were collected from the ventricles to allow meaningful comparisons of CSF smooth-muscle constrictor activity within outcome groups. However, lumbar and ventricular CSF samples were obtained from three patients (Cases 3, 6, and 17). For the values in those patients see Table 3. The only major difference is seen with Case 3, where a high value for smooth-muscle constrictor activity in ventricular CSF, sampled 7 days after lumbar CSF was obtained, coincided with the onset of delayed ischemic deficits.

**Correlation with Angiographic Vasospasm.** The mean smooth-muscle constrictor activity detected in lumbar and ventricular CSF from 32 SAH patients was 21.2 ± 9.6 nmol/liter PGE\(_2\) equivalents for 500 μl of CSF in a 5-ml bath (Tables 1 and 2, and Fig. 1). This was considerably higher than that measured in CSF from six control patients (1.17 ± 0.34 nmol/liter PGE\(_2\) equivalents). Among the SAH patients, the mean constrictor activity in the seven cases with severe angiographic vasospasm (67.0 ± 41.0 nmol/liter PGE\(_2\) equivalents) was 10 times greater than that seen in the 19 patients in whom angiography did not demonstrate severe vasospasm (6.7 ± 1.7 nmol/liter PGE\(_2\) equivalents, p < 0.01). There was no apparent graded relationship between concentration of smooth-muscle constrictor material in the CSF and the severity of angiographic vasospasm. The groups of patients with mild and moderate angiographic spasm (Table 2) were, however, very small.

**Correlation with Outcome.** The greatest concentrations of vasoconstrictor material were seen in CSF samples from patients who died with evidence of delayed ischemic deficits (73.8 ± 39.7 nmol/liter PGE\(_2\) equivalents). This was compared with values in the CSF from patients with a poor outcome (6.7 ± 2.9 nmol/liter PGE\(_2\) equivalents, p < 0.1), from those with a fair outcome (4.2 ± 1.0 nmol/liter PGE\(_2\) equivalents, p < 0.2), and from those with a good outcome (6.8 ± 1.8 nmol/liter PGE\(_2\) equivalents, p < 0.01). Ranking the controls as 0, good outcome as 1, fair outcome as 2, poor outcome as 3, and death as 4, there was a slight positive correlation (r = 0.74) between outcome and CSF smooth-muscle constrictor activity.

Serial lumbar CSF samples obtained from one patient (Case 2) were tested on both the rat fundus and the isolated human basilar artery. There was good agreement between the values for smooth-muscle constrictor activity estimated by these preparations (Fig. 2). Increasing activity was associated with angiographic vasospasm, the onset of delayed ischemic deficits, and subsequent death.

**TABLE 3**

<table>
<thead>
<tr>
<th>Source of CSF</th>
<th>Case 3</th>
<th>Case 6</th>
<th>Case 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>lumbar CSF</td>
<td>3.2</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>day after SAH</td>
<td>5</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>ventricular CSF</td>
<td>74.0</td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td>day after SAH</td>
<td>12</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

* Smooth-muscle constrictor activity is given in nmol/liter PGE\(_2\) equivalents. CSF = cerebrospinal fluid; SAH = subarachnoid hemorrhage.

**FIG. 1.** Relationship between concentration of smooth-muscle constrictor substances in cerebrospinal fluid (CSF) and clinical outcome. Individual bioassay values (isolated rat fundus, closed circles) are plotted against outcome. Mean values ± SE are given for each group (closed squares). The greatest concentrations of vasoconstrictor material were seen in the CSF obtained from patients who subsequently died with evidence of vasospasm (see Tables 1 and 2). For a definition of outcome see text.
Discussion

Numerous in vivo and in vitro methods for the study of cerebral vasospasm have been developed. While primate models might seem to be most suitable for such research, cost precludes their use in most centers. In vivo angiographic techniques using small rodents have been developed, but, as with other live animal models, there are problems with inter-experiment variability and sample capacity. Of the in vitro models for investigating the effects of smooth-muscle constrictors on the human cerebral vasculature, the isolated human intracranial artery seems, at first, most suitable. However, a recent report has highlighted the variability of such preparations, making accurate, comparable bioassays of a large number of CSF samples difficult. Accordingly, the isolated rat stomach fundus was chosen for estimation of smooth-muscle constrictor activity. Boulin, et al., have shown that the responses of this preparation to a wide range of physiological fluids (including post-SAH CSF) are very similar to the responses obtained from isolated human cerebral vessels. Furthermore, good correlations have been demonstrated between smooth-muscle constrictor activity of serial CSF samples (obtained after SAH), when assayed on both isolated rat fundus and isolated human basilar artery preparations. Similar results were seen with serial samples of CSF from Case 2 (Table 2 and Fig. 2). Recent research has also shown similar profiles of serotoninergic receptor response to post-SAH CSF, for both preparations. Accordingly, the rat stomach preparation was chosen for maximum reproducibility of bioassay, using serotonin and PGE₂ as external standards of smooth-muscle constrictor compounds.

No significant differences were seen between the concentration of smooth-muscle constrictor material in lumbar CSF and that in ventricular CSF. Although the number of patients studied was small, this suggests that significant gradients of vasoactive materials may not occur in the CSF after SAH in the way that tryptophan metabolite gradients occur in patients without evidence of subarachnoid blood. A similar lack of a ventricular/lumbar gradient has recently been demonstrated for concentrations of serotonin in CSF from patients after aneurysm rupture. Fisher, et al., in a study of 50 SAH cases, reported delayed ischemic deficits in 25 patients, all of whom showed severe angiographic spasm. However, severe vasospasm was reported in six patients without concomitant ischemic deficits. Similar results were seen in the present study. Three of our seven patients who died (Cases 1, 2, and 3, Table 2) showed severe angiographic vasospasm and ischemic deficits (angiography was not performed in Cases 4 to 7). However, three patients with a good outcome (Cases 14, 15, and 16, Table 2) showed severe arterial narrowing at angiography, but no associated neurological deficits.

Angiography was clinically justified in only 26 out of 32 cases. There were insufficient numbers of patients with either mild or moderate vasospasm to demonstrate a graded relationship between angiographic vasospasm and smooth-muscle constrictor activity in CSF. However, the mean concentration of smooth-muscle constrictor material was significantly greater in seven cases with severe angiographic arterial narrowing compared with 19 patients without angiographic spasm. The value of these data is strengthened by the fact that the mean time of CSF collection (11.1 ± 1.5 days) was not significantly different from the mean time of angiography (10.9 ± 1.1 days) for all patients.

Analysis of the data in Table 2 and Fig. 1 shows that the concentrations of smooth-muscle constrictor sub-

![Graph of smooth-muscle constrictor material in CSF](image-url)
stances in the CSF of patients who subsequently died was significantly elevated in comparison with all other SAH patients (p < 0.01, two-tailed unpaired t-test), despite the small size of the group and the wide range of the values measured. Values were also elevated in comparison with those obtained in patients with a "poor" outcome and residual neurological deficits, but less significantly so (p < 0.1). In one patient with severe angiographic vasospasm and delayed ischemic deficits (Case 2, Table 2 and Fig. 2), CSF smooth-muscle constrictor activity increased from 191.2 nmol/liter PGE2 equivalents on Day 7 to 302.4 nmol/liter PGE2 equivalents on Day 14. Focal ischemic deficits were observed on Day 8, and slowly increased in severity until death occurred 10 days later. This suggests a link between spasm, pharmacological activity, and outcome.

There were some anomalous results in terms of the concentration of smooth-muscle constrictor activity and both outcome (Cases 7 and 21) and angiographic vasospasm (Cases 16 and 21); however, there was a slight correlation (r = 0.74) between pharmacological activity of CSF and ranked outcome, despite the wide range of values in the group of patients who subsequently died. The lack of an obvious graded relationship between smooth-muscle constrictor substances in the CSF and clinical outcome is not surprising. Many factors may contribute to the clinical state, including high intracranial pressure, intracerebral clots, and focal exacerbations of cerebral vasospasm which may not be visualized by cerebral angiography, even if performed at the appropriate time. However, the slight correlation detected suggests that a vasoconstrictor stress exerted by unidentified smooth-muscle constrictor substances in the CSF may affect recovery from ruptured aneurysms, possibly by acting on small vessels which cannot be visualized angiographically.

Cerebral arteries, both in vivo and in vitro, have been shown to be responsive to a very large range of physiologically occurring substances. These include angiotensin II,26 serotonin,12,14,16 potassium,13,18 thrombin,19,42,43 vasopressin,26,27 prostaglandins E2 and F2α,45 histamine,25 leukotrienes,35 norepinephrine,4,34 hemoglobin,12,32 and lipid peroxides.30 Furthermore, unidentified vasoconstrictor substances have been isolated from incubates of fibrinogen,17 platelets,23 erythrocytes,28 and blood/CSF mixtures.33

Of all these substances, only vasopressin,26 hemoglobin,36 and serotonin36,41 have been detected in increased concentrations in CSF obtained from patients after SAH. None of these studies was able to demonstrate angiographic and clinical spasm resulting in death, to attempt a separation and characterization of pharmacologically active substances. However, a previous study at this center8 demonstrated that contractions of smooth-muscle preparations elicited by CSF obtained from SAH patients were not mediated by receptors for serotonin, histamine, norepinephrine (α and β), acetylcholine, or angiotensin.

The origin of the smooth-muscle constrictor substances appearing in CSF remains obscure. It is possible that they are released by damaged brain tissue, or they may be derived from blood.33 The relationship between the degree of CSF/blood admixture and concentration of smooth-muscle constrictor material in the CSF was not investigated in the present study, due to the difficulty of obtaining reliable hematocrit measurements in the presence of cell debris and clot material.

Previous investigations of the clinical pharmacology of cerebral vasospasm at this center5,8,11 have focused on the relationship between smooth-muscle constrictor substances in CSF collected postoperatively, and subsequent angiographic spasm. One study21 was extended to the preoperative period, and CSF activity generally declined throughout the period of hospitalization (up to 43 days after SAH), but increased in cases showing sudden clinical deterioration with development of ischemic deficits. A similar result was seen with Case 2 (Table 2 and Fig. 2).

The presence of high concentrations of CSF smooth-muscle constrictor activity in patients who subsequently died of their disease, and of a positive correlation (r = 0.74) between the pharmacological activity of the CSF and ranked outcome, suggests that bioassay of preoperatively collected CSF might be used to detect patients at greatest risk. There could also be a case for serial lumbar punctures in selected patients to determine if CSF activity is increasing or declining.

However, the occurrence of highly active CSF in the absence of clinical deficits or angiographic evidence of spasm in an isolated instance (Case 21), and low concentrations of constrictor material in the CSF of two patients who died (Cases 6 and 7), implies that the relationship between vasospasm and pharmacological activity is not straightforward. Nevertheless, the data indicate that CSF sampling for assessment of smooth-muscle constrictor activity might be clinically useful in selected patients after aneurysm rupture.

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