Leukotrienes derive from arachidonic acid metabolism via the lipoxygenase pathway and modulate several cellular events. In the central nervous system, leukotrienes are mainly synthesized in the gray matter and in vascular tissues. Their production is enhanced in ischemic conditions and in experimental subarachnoid hemorrhage (SAH). Previous studies have indicated the ability of the leukotrienes C₄ and D₄ to constrict arterial vessels in vivo and in vitro and have suggested their involvement in the pathogenesis of cerebral arterial spasm. In the present study, the authors measured lumbar and cisternal cerebrospinal fluid (CSF) levels of leukotriene C₄ in 48 patients who had suffered aneurysmal SAH. In 12 of the cases, symptomatic and radiological spasm was evident. The mean lumbar CSF level of immunoreactive-like activity of leukotriene C₄ (i-LTC₄) was significantly higher (p < 0.005) than in control cases, while the cisternal CSF level was higher than the lumbar mean concentration (p < 0.005). Patients presenting with vasospasm had significantly higher levels of i-LTC₄ compared to patients without symptomatic vasospasm.

This is the first report concerning monitoring of i-LTC₄ levels in the CSF after SAH. The results of this study suggest that: 1) metabolism of arachidonic acid via the lipoxygenase pathway is enhanced after SAH; 2) the higher cisternal CSF levels of i-LTC₄ may be part of the biological response in the perianeurysmal subarachnoid cisterns after the hemorrhage; and 3) the higher CSF levels of i-LTC₄ in patients presenting with vasospasm suggest that a relationship exists between this compound and arterial spasm and/or reflect the development of cerebral ischemic damage.

KEY WORDS • subarachnoid hemorrhage • vasospasm • leukotriene • cerebral ischemia
CSF leukotriene C₄ and subarachnoid hemorrhage

...tion is enhanced immediately after experimental SAH consonant with the extent of subarachnoid blood diffusion and possibly with the vasospasm-related cerebral ischemic damage.

The purpose of the present study was to measure cerebrospinal fluid (CSF) levels of immunoreactive-like activity of the leukotriene C₄ (i-LTC₄) in patients admitted with a diagnosis of aneurysmal SAH and to discuss the possible role of leukotrienes in the pathophysiological aspects of the disease.

Clinical Material and Methods

Patient Population

Lumbar and cisternal CSF samples were collected from 61 selected good-risk patients with SAH. Clinical diagnosis was made by means of computerized tomography (CT) and lumbar puncture. Clinical assessment was performed according to the grading system of Hunt and Hess. All of the patients were treated with tranexamic acid (6 gm/day intravenously for 15 days) and anticonvulsant agents. Patients were selected for inclusion in the study by the following criteria: 1) they were in good clinical condition (Hunt and Hess Grade I to III on admission); 2) no steroids had been administered; and 3) a lumbar CSF sample had been collected between Days 1 and 3 after SAH. Angiography was not performed before Day 8 after SAH (late-surgery protocol). The diagnosis of vasospasm was made upon the appearance of clinical signs (reduced level of consciousness and/or focal neurological deficit) and by CT evidence of focal ischemia without rebleeding. Computerized tomography was always repeated before surgery. Cisternal CSF samples were obtained by intraoperative cisternal puncture. Control lumbar CSF samples were obtained during myelography from patients with intravertebral pathology. For ethical reasons, cisternal CSF samples were not available from control patients.

Measurement of Leukotriene C₄

The CSF samples were collected and immediately placed in ice (−4°C) in order to minimize in vitro residual release of arachidonic acid metabolites. The samples were then centrifuged (6500 rpm for 5 minutes at 4°C), frozen in liquid nitrogen, and stored (−80°C) until analysis. In these conditions the whole-cell component responsible for residual release of i-LTC₄ is separated and the CSF levels of this compound could be reasonably evaluated, avoiding in vivo residual production of the metabolite.

The i-LTC₄ activity was detected by the radioimmunoassay technique of Levine, et al., using an antiserum* to the leukotriene C₄ which has a cross-reactivity of 10.1% with the leukotriene D₄, 2.3% with the leukotriene E₄, 0.07% with hydroxyeicosatetraenoic acid (HETE), and 0.006% with the leukotriene B₄. Ten milliliters of Atomlight high sample capacity scintillation solution (NEF-968) was added to each sample.† Radioactivity was measured using a liquid scintillation counter.‡ The i-LTC₄ activity is expressed in picograms per milliliter of CSF. The assay sensitivity was 6 pg/ml of CSF. Results were compared by Student's t-test for unpaired data.

Results

Of 61 patients entered into the study, 48 had single or multiple aneurysms and 13 were classified as having an SAH of unknown origin. The general characteristics of this patient population are summarized in Table 1 and their CT classification is given in Table 2. Lumbar CSF samples were available in 48 cases of aneurysmal SAH. In 36 patients the clinical course was good and uncomplicated by cerebral vasospasm, whereas 12 patients had clinical and radiological evidence of vasospasm. Cisternal CSF samples were available in the 12 patients with a diagnosis of vasospasm and in 30 of the 36 patients without vasospasm.

The mean (± standard error of the mean) lumbar CSF level of i-LTC₄ in all 48 patients with aneurysmal

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* Antiserum to leukotriene C₄ supplied by New England Nuclear Chemicals GmbH, Dreieich, West Germany.

† Atomlight scintillation solution supplied by United Technologies Packard, Packard Instruments, Downers Grove, Illinois.

‡ Tri-carb scintillation spectrometer, Model 3320, manufactured by Packard Instruments, Downers Grove, Illinois.
Despite their common precursor, the two different pathways of arachidonic acid metabolism act differently in cerebral pathophysiological responses after aneurysmal SAH. The metabolites via the cyclo-oxygenase pathway are primarily vasoactive agents and modulate arterial tone, whereas lipoxygenase metabolites mostly participate in modifications of membrane permeability, brain edema formation, and local inflammatory processes. Recently published experimental studies have demonstrated increased production of leukotrienes in the brain in pathological conditions such as head injury, cerebral ischemia, and SAH. No published data are available concerning leukotriene detection in the human CSF after SAH. This study showed that, after rupture of the aneurysm, there was a marked increase in the lumbar CSF levels of i-LTC₄. The different clinical outcome (that is, vasospasm or no vasospasm) in patients in whom the same medical treatment was administered could be attributed to a specific enhancement of arachidonic acid metabolism after SAH and is in agreement with experimental observations. Lumb 4 Lumbar CSF levels of i-LTC₄ were significantly lower than in cisternal samples collected 10 to 12 days after SAH in the perianeurysmal cisterns. Leukotriene production is enhanced by contact between cisternal clot and neuronal tissue (although the vascular synthesis must also be taken into account), and it persists until blood breakdown reactions are completed.

A possible explanation for the higher lumbar CSF levels of i-LTC₄ could be the preoperative treatment with tranexamic acid. This treatment could interfere with cisternal clot dissolution, favor the release of arachidonic acid metabolites, and promote a prolonged exposure of the arterial wall to vasoactive compounds. The different clinical outcome (that is, vasospasm or no vasospasm) in patients in whom the same medical treatment was administered could be attributed to a different pattern of i-LTC₄ production by brain tissue. The subarachnoid deposition of blood appears to be spread thinner in cases of SAH of unknown origin, as classified by the CT findings. Aneurysmal SAH would sustain a characteristic pathophysiological event with a higher leukotriene production in response to a larger subarachnoid deposition of blood, as classified by CT findings (Table 4); this could confirm experimental evidence that leukotriene production is dependent upon

**Discussion**

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**Table 3**

<table>
<thead>
<tr>
<th>Levels of i-LTC₄</th>
<th>CSF Source</th>
<th>Control Cases</th>
<th>Aneurysmal SAH</th>
<th>SAH of Unknown Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of cases</td>
<td></td>
<td>12</td>
<td>48</td>
<td>13</td>
</tr>
<tr>
<td>lumbar</td>
<td>92.54 ± 16.28</td>
<td>334.81 ± 53.56</td>
<td>161.92 ± 15.18</td>
<td></td>
</tr>
<tr>
<td>cisternal</td>
<td>1588.69 ± 331.90</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cerebrospinal fluid (CSF) levels of immunoreactive-like activity of leukotriene C₄ (i-LTC₄) are mean values ± standard error of the mean. Student's t-test for unpaired data was used for comparing results. Significance: p < 0.005, aneurysmal subarachnoid hemorrhage (SAH) vs. control cases; p < 0.02, aneurysmal SAH vs. SAH of unknown origin; p < 0.005, cisternal vs. lumbar CSF level of i-LTC₄ in aneurysmal SAH.

**Table 4**

<table>
<thead>
<tr>
<th>Levels of i-LTC₄</th>
<th>Time of CT Scanning</th>
<th>Lumbar CSF sample</th>
<th>Cisternal CSF sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of cases</td>
<td>CT Group I</td>
<td>CT Groups II &amp; III</td>
<td></td>
</tr>
<tr>
<td>at admission</td>
<td>300.42 ± 52.2</td>
<td>288.54 ± 43.62</td>
<td></td>
</tr>
<tr>
<td>preoperatively</td>
<td>685.29 ± 115.43</td>
<td>2044.04 ± 499.23</td>
<td></td>
</tr>
</tbody>
</table>

* Cerebrospinal fluid (CSF) levels of immunoreactive-like activity of leukotriene C₄ (i-LTC₄) are mean values ± standard error of the mean. Student's t-test for unpaired data was used for comparing results. Significance: † = p < 0.02 when compared to computerized tomography (CT) Group I. SAH = subarachnoid hemorrhage.
CSF leukotriene C₄ and subarachnoid hemorrhage

contact between blood in the subarachnoid space and brain tissue.²⁰

In our study, lumbar and cisternal CSF levels of i-LTC₄ were significantly higher in patients presenting with symptomatic vasospasm (Table 5). The vasoactive properties of leukotrienes have been investigated in different experimental conditions.³² ³⁵ ³⁸ ³⁹ The fact that the highest level of i-LTC₄ is detected in the cisternal CSF of patients presenting with vasospasm suggests that the leukotriene C₄ may be a possible vasoconstricting agent. Leukotrienes interact with other eicosanoids and mainly inhibit prostacyclin synthesis; this could result in a lower defensive mechanism of the arterial endothelium against other vasoconstricting agents.²³

In addition to the above-mentioned findings, an increased biosynthesis of leukotrienes in brain tissue has been reported in experimental ischemia.⁴ ⁷ ²⁵ The higher cisternal CSF levels of i-LTC₄ in patients with demonstrated vasospasm could also be related to regional cerebral hypoperfusion, which enhances neuronal production of the compound. Another question concerns white blood cell (WBC) production of leukotrienes.

Recently, Yokota, et al.,⁴² showed that pharmacological inhibition of lipooxygenase could prevent, but not reverse, delayed vasospasm in experimental SAH. The presence of inflammatory cells in the tunica media and in the adventitia of arteries of patients who died of SAH and vasospasm has also been reported;¹⁰ ¹⁶ these cells could be the source of leukotrienes² ³⁷ and other vasoactive compounds that participate in microcirculatory regulation.²⁴ ⁴¹

The elevated WBC count in CSF after hemorrhage could be the expression of an enhanced local inflammatory response to the hemorrhage; moreover, the correlation between an initial serum WBC count higher than 20,000 cells/ml of CSF and clinical outcome, with special reference to vasospasm, has been noted as a possible prognostic factor.²⁶ ²⁷ In our series, the mean WBC count at admission was 12,635.7 ± 1538.3 cells/ml of peripheral blood in patients with a good and uncomplicated clinical outcome (no vasospasm), whereas it was 16,214.3 ± 2374.8 cells/ml of peripheral blood in patients presenting with vasospasm: the difference is not statistically significant. The mean WBC counts in lumbar CSF at admission did not correlate well with the incidence of vasospasm (Table 6), nor with the lumbar CSF level of i-LTC₄; this suggests that the granulocyte production of the compound accounts for only a small part. The local inflammatory process enhances leukotriene production either from WBC's in perianeurysmal cisterns or from brain tissue. In our series, the mean WBC count in cisternal CSF (10 to 12

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days after SAH) was very low (<1 cell/ml), suggesting that arachidonic acid metabolism via the lipoxygenase pathway in brain tissue plays a primary role. An experimental design in rats with SAH is now being investigated in an attempt to clarify this.

Every possible action of leukotrienes on brain structures should be evaluated on the basis of its quality. Are leukotrienes the mediators of a kind of pathological damage, or is leukotriene release in CSF a part of a tonic response of the brain in the accumulation of arachidonic acid during posts ischemic or post-hemorrhagic hypoperfusion? There is no definite answer to this question at the moment.

In our study, a prolonged release of i-LTC4 was suspected from observation of cisternal CSF samples collected on Day 10 or later after the hemorrhage. In the early post-hemorrhagic phase there was no significant difference in the lumbar CSF level of i-LTC4, if related to CT classification (Table 4), whereas the cisternal CSF level of i-LTC4 was significantly higher in patients with a larger amount of blood as shown on preoperative CT scans. The amount of subarachnoid blood deposited is known to be associated with the risk of vasospasm.11

In conclusion, changes in the CSF level of i-LTC4 after SAH and vasospasm onset suggest a role for leukotrienes in the pathogenesis of cerebral arterial spasm. Hypothetically, the generalized hypoperfusion induced by the hemorrhage may enhance the release of arachidonic acid metabolites and promote leukocyte and macrophage adhesion in the subarachnoid space and in the arterial adventitia. Leukotrienes in cisternal CSF may act as a spasmogen and inhibit vasodilating factors of the arterial wall, such as prostacyclin. Vasospasm, by itself, induces brain hypoxia and the further release of spasmodgens which could sustain a self-maintaining reaction leading to delayed vasospasm.

The results of this clinical study confirm experimental evidence of enhanced arachidonic acid metabolism via the lipoxygenase pathway after SAH and suggest the important role of i-LTC4 in determining local brain responses after the aneurysm rupture.

TABLE 6

<table>
<thead>
<tr>
<th>WBC Source</th>
<th>WBC Count</th>
<th>Vasospasm</th>
<th>No Vasospasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood (at admission)</td>
<td>16,214.3 ± 2374.8</td>
<td>12,635.7 ± 1538.3</td>
<td></td>
</tr>
<tr>
<td>lumbar CSF (at admission)</td>
<td>54.7 ± 5.3</td>
<td>53.6 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>cisternal CSF (at surgery)</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td></td>
</tr>
</tbody>
</table>

* White blood cell (WBC) values are means ± standard error of the mean and are measured in cells per milliliter in both peripheral blood and cerebrospinal fluid (CSF). Student's t-test for unpaired data was used for comparing results: the differences between the samples from the two groups were not statistically significant.

References

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Acknowledgment

The authors gratefully acknowledge the technical assistance of Mrs. I. Fugaccia.

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Manuscript received January 4, 1988.
This work was supported in part by a Grant from the Regione Lombardia, Milan, and in part by a grant from the Italian Ministry of Public Education, Rome.
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