Detection of meningeal fibrosis after subarachnoid haemorrhage by assaying procollagen propeptides in cerebrospinal fluid

Juha Sajanti, Kari Majamaa

Abstract

Objective—To study whether meningeal collagen synthesis under normal conditions is reflected in the CSF and whether a meningeal fibroproliferative reaction or fibrosis after subarachnoid haemorrhage can be detected by measuring markers of collagen synthesis in the CSF.

Methods—Serum samples and CSF were collected from 56 patients with various neurological symptoms and from nine patients with a recent subarachnoid haemorrhage. The concentrations of the carboxyterminal propeptide of type I procollagen (PICP) and the aminoterminal propeptide of type III procollagen (PIIINP) were measured using radioimmunoassays.

Results—The mean (SD) concentration of PICP was 75.2 (SD 13.6) µg/l and that of PIIINP 3.56 (SD 0.91) µg/l in the CSF of the controls, and the CSF/serum ratios were 0.74 (SD 0.24) for PICP and 1.34 (SD 0.48) for PIIINP. A 1.4-fold increase in both the PICP (p=0.001) and the PIIINP (p=0.001) concentration was found in patients with a neurological disease and with an abnormal CSF leucocyte count or protein concentration. In eight patients with a recent subarachnoid haemorrhage the PICP was 5.9-fold higher (p<0.001) and the PIIINP concentration 7.7-fold higher (p<0.001) than in the controls, whereas no difference was found in the serum values. Similar high concentrations were also found in a patient from whom the CSF sample was obtained before operation for aneurysm.

Conclusions—The intrathecal compartment is a site for active collagen synthesis under normal conditions. The synthesis rate is markedly increased in patients with a recent subarachnoid haemorrhage, suggesting a fibroproliferative reaction or fibrosis. Assays of procollagen propeptides may be useful in the clinical diagnosis of meningeal fibrosis and their use may enable the identification of diseases and symptoms aetiologically related to meningeal fibrosis.

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Keywords: meninges; collagen; CSF protein; laboratory analysis

A significant proportion of the extracellular matrix of the dura and the arachnoid is composed of different collagens that are synthesised by dural fibroblasts or leptomeningeal cells. Mesenchymal cells such as these may be provoked by noxious stimuli to increase their synthesis of the extracellular matrix components, resulting in fibrosis. Meningeal fibrosis has been seen histologically in experimental animals after the injection of blood into the subarachnoid space and in patients who died from subarachnoid haemorrhage. These findings suggest that the meninges are a site for a fibroproliferative reaction as well as fibrosis in subarachnoid haemorrhage. Clinically, however, meningeal fibrosis is a poorly defined entity, although diffuse meningeal enhancement seen on MRI may suggest the diagnosis.

The synthesis of collagens involves many enzyme catalysed modification reactions both inside and outside the cell. The activity of some of these enzymes in tissue samples or the contents of some of the reaction products in tissue fluids have been used as an indicator of the rate of collagen biosynthesis both in normal conditions and in fibrosis. Useful clinical markers of collagen synthesis seem to be measurement of the concentrations of the C-terminal propeptide of type I procollagen (PICP) and the N-terminal propeptide of type III procollagen (PIIINP) in serum.

Analysis of collagen synthesis in CSF would introduce higher sensitivity in the diagnostics of meningeal fibroproliferative states compared with imaging studies, but the applicability of this approach has not been assessed. Therefore, we evaluated practical methods for studying the rate of collagen synthesis in the intrathecal compartment. We measured in the CSF the activity of two enzymes that are involved in the intracellular processing of collagens and the concentration of two procollagen propeptides that are released into the extracellular space during biosynthesis.

Patients and methods

PATIENTS

A CSF sample and a parallel serum sample from 32 consecutive patients undergoing a diagnostic spinal tap was collected. After the diagnostic evaluation had been completed, the patients were considered for inclusion in one of two clinically defined subgroups. The first subgroup—group 1 controls—comprised 21 patients (headache, six; dizziness or vertigo, five; sensory symptoms, three; depression, two; psycho-organic syndrome, one; memory impair-
An aneurysm was found and operated on in each patient (anterior communicating artery, four; medial cerebral artery, four; posterior cerebral artery, one). A CSF sample from one patient was obtained before aneurysm operation at day 18 after the bleeding. This patient is not included in the data on the timing of the aneurysm operation.

The study protocol has been approved by the ethics committee of the Medical Faculty of the University of Oulu. The samples were obtained with the informed consent of the patients.

METHODS

The CSF and blood samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was stored at −75°C until assayed. The cell count of the CSF was determined as part of the routine clinical chemical analysis of the CSF. The total protein content was determined using a colorimetric assay.12

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### Table 1 Clinical features of patients with subarachnoid haemorrhage (group 2)

<table>
<thead>
<tr>
<th>Clinical grade on admission</th>
<th>Patient group</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunt and Hess</td>
<td>Matched</td>
<td>2</td>
<td>1–3</td>
</tr>
<tr>
<td>Amount of blood in CT</td>
<td>Group 1</td>
<td>18</td>
<td>2–22</td>
</tr>
</tbody>
</table>

Clinical events after the bleeding

| First CT (h) | Group 1 | 4.8   | 1.9–132 |
| Aneurysm operation* (days) | Group 1 | 3.5   | 0.5–8   |
| CSF sample for analysis (days) | Group 1 | 14    | 8–25   |

An aneurysm was found and operated on in each patient (anterior communicating artery, four; medial cerebral artery, four; posterior cerebral artery, one).

*CSF sample from one patient was obtained before aneurysm operation at day 18 after the bleeding. This patient is not included in the data on the timing of the aneurysm operation.

### Table 2 Clinical features of patients in group 1 and group 2 and their PICP and PIIINP concentrations in CSF and serum

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Group 1 controls</th>
<th>Group 1 patients</th>
<th>Group 2 patients</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>CSF protein (mg/l)</td>
<td>320 (115)</td>
<td>741 (397)†</td>
<td>377–1974</td>
</tr>
<tr>
<td></td>
<td>PICP (µg/l)</td>
<td>75.2 (13.6)</td>
<td>103 (19.2)‡</td>
<td>175–898</td>
</tr>
<tr>
<td></td>
<td>In CSF (µg/l)</td>
<td>3.56 (0.91)</td>
<td>5.14 (1.65)†</td>
<td>11.1–74.6</td>
</tr>
<tr>
<td></td>
<td>In serum (µg/l)</td>
<td>2.85 (0.78)</td>
<td>3.41 (1.23)</td>
<td>1.05–5.52</td>
</tr>
<tr>
<td></td>
<td>PIIINP (µg/l)</td>
<td>2.85 (0.78)</td>
<td>3.41 (1.23)</td>
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</tr>
</tbody>
</table>

### Results

Preliminary studies on the CSF and serum samples from 24 separate patients with a neurological disease and with abnormal CSF suggested that the amount of aminoterminal propeptide of type III procollagen (RIA-gnost PIIIP; Behringwerke, Marburg, Germany) and the activity of collagen glucosyltransferase were readily measurable in the CSF, whereas the activity of prolyl 4-hydroxylase was undetectable. The data also suggested that the assay of the procollagen propeptide is more sensitive than that of collagen glucosyltransferase. Therefore, the studies reported here were carried out using specific radioimmunoassays for both the aminoterminal propeptide of type III procollagen (PIIINP) and for the carboxyterminal propeptide of type I procollagen (PICP) (Orion Diagnostica, Turku, Finland).

#### STATISTICAL ANALYSIS

The normality of the distributions was verified first by using both the Kolmogorov-Smirnov test with Lilliefors’ significance correction and the Shapiro-Wilk test. In most of the groups the values were normally distributed and therefore statistical analysis was carried out by one way analysis of variance (ANOVA) to detect the differences between the groups and subsequent comparisons between the two groups were made by paired or unpaired t tests, as appropriate. Pearson’s correlation coefficient was calculated when the concentrations of the propeptides in the CSF were compared with the total protein.

#### CONCENTRATIONS OF PICP AND PIIINP IN THE CSF AND SERUM OF THE CONTROLS AND PATIENTS WITH NEUROLOGICAL SYMPTOMS AND WITH AN ABNORMAL CSF

We assayed CSF and serum samples for the concentrations of PICP and PIIINP from 32 patients (group 1). The concentration of PICP in the CSF of group 1 controls was 75.2 (13.6) µg/l and that of PIIINP was 3.56 (0.91) µg/l (table 2). We did not find any changes with age or sex in these concentrations. The CSF/serum ratio was 1.34 (95% confidence interval (95% CI) 1.12–1.56) for PIIINP, and the corre-

### Table 2 Clinical features of patients in group 1 and group 2 and their PICP and PIIINP concentrations in CSF and serum

<table>
<thead>
<tr>
<th>Clinical description</th>
<th>Patient group</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF protein (mg/l)</td>
<td>No CNS disease</td>
<td>741 (397)†</td>
<td>777–1974</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>Neurological disease with abnormal CSF</td>
<td>103 (19.2)‡</td>
<td>175–898</td>
</tr>
<tr>
<td>CSF protein (mg/l)</td>
<td>Neurological disease with abnormal CSF</td>
<td>126 (35.0)</td>
<td>1.05–5.52</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>Neurological disease with abnormal CSF</td>
<td>126 (35.0)</td>
<td>1.05–5.52</td>
</tr>
<tr>
<td>PIIINP (µg/l)</td>
<td>Neurological disease with abnormal CSF</td>
<td>126 (35.0)</td>
<td>1.05–5.52</td>
</tr>
<tr>
<td>CSF protein (mg/l)</td>
<td>Recent SAH</td>
<td>995 (514)¶</td>
<td>777–1974</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>Recent SAH</td>
<td>126 (35.0)</td>
<td>1.05–5.52</td>
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*p<0.05; †p<0.01; ‡p=0.001; ¶p<0.001; ND=not determined.
spending ratio for PICP was 0.74 (95% CI 0.63–0.85). The PICP/PIIINP ratio in the serum was twofold higher than that in the CSF. There was a significant correlation of PICP in the CSF with PIIINP in the CSF (r = 0.832; p < 0.001). On the other hand, the CSF concentrations of the two propeptides did not correlate with the total CSF protein concentration or to the serum concentrations of the respective propeptides.

Group 1 patients included 11 persons with a neurological disease or neurological symptoms and with an abnormal CSF leucocyte count or protein content (table 2). The concentrations of both PICP and PIIINP in the CSF of these patients were 1.4-fold higher than those of the controls (p = 0.001). We did not find differences in the serum values nor in the various ratios that were calculated (table 2).

MARKEDLY INCREASED INTRATHECAL COLLAGEN SYNTHESIS IN PATIENTS WITH A RECENT SUBARACHNOID HAEMORRHAGE

Markedly increased concentrations of PICP and PIIINP in the CSF were found in nine patients with a recent subarachnoid haemorrhage (group 2), whereas no increases were found in the corresponding serum concentrations (table 2). The mean concentration of PICP in the CSF of group 2 was 5.9-fold higher and that of PIIINP 7.7-fold higher than in controls. The corresponding CSF/serum ratios were increased in a similar manner. The CSF sample from one patient in group 2 was obtained before aneurysm operation and similar high concentrations of the propeptides were also found in this patient. The PICP/PIIINP ratio in the CSF and the serum was not different between group 2 and the controls. The increase in the total protein content of CSF was only 3.1-fold in group 2 and, furthermore, we did not find a correlation between the two propeptide concentrations and the total protein concentration.

Discussion

The propeptides of type I and type III procollagens were found to belong to a group of rare proteins, the concentration of which is disproportionately high in the CSF compared with that in the serum. In general, the CSF/serum ratios of the major serum proteins are low—for example, that of albumin is around 1:200—due to the function of the blood-CSF barrier. Proteins with a disproportionately high CSF concentration include transthyretin (prealbumin), prostaglandin D synthase (the β-trace protein), cystatin C (the γ-trace protein), and transferrin. With the exception of transferrin, active synthesis of these proteins in the choroidal epithelium or elsewhere in the CNS has been shown in humans. We calculated that passive diffusion across the blood-CSF barrier could account for less than 1% of the procollagen propeptides in the CSF suggesting an active collagen turnover in the intrathecal compartment.

The diagnosis of meningial fibrosis is usually a radiological or histological one, and markers of collagen metabolism have been seldom measured in the CSF. We found minor but sig-


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