Determination of S-100 and Glial Fibrillary Acidic Protein Concentrations in Cerebrospinal Fluid After Brain Infarction

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Background and Purpose: We initiated the present study to evaluate the clinical value of consecutive concentration determinations of S-100 and glial fibrillary acidic proteins in cerebrospinal fluid from patients with brain infarction.

Methods: We took sequential samples of cerebrospinal fluid from 28 patients within 48 hours, at 7 days, and at 18–21 days after the ictus. We measured astroglial protein concentrations using an enzyme-linked immunosorbent assay and also determined size of the infarction (computed tomography), clinical state of the patient (simplified activities of daily living test), blood–brain barrier dysfunction (cerebrospinal fluid/serum albumin ratio), and a myelin marker (myelin basic protein).

Results: We found a transient increase of both proteins in the cerebrospinal fluid during the first week after the ischemic stroke (p<0.05). This increment was significantly correlated with the size of the infarction and the clinical state of the patients.

Conclusions: Transient release of astroglial proteins into the cerebrospinal fluid possibly reflects initial focal ischemic damage and, in the later phase, ongoing destruction of astroglial cells in the penumbra zone. We suggest that determinations of cerebrospinal fluid astroglial protein concentrations can be used to estimate ischemic brain damage, which should be of particular value in clinical trials of pharmacological agents, such as calcium antagonists, on stroke patients.

Stroke is one of the most common causes of death in Western countries, with roughly 80% due to cerebral ischemia. Cerebral infarction causes necrosis of a part of the central nervous system, and the sequelae vary according to the localization of the disease process and the extent of cerebral damage.

The S-100 and glial fibrillary acidic (GFA) proteins are synthesized in astroglial cells in all parts of the central nervous system. Because only low levels of these proteins are present in serum, structural damage to the brain causes a selective leakage of the S-100 and GFA proteins from the brain tissue into the cerebrospinal fluid (CSF) irrespective of the blood–brain barrier dysfunction.

The low molecular weight and high degree of solubility of S-100 make this protein a suitable CSF marker for damage to cerebral tissue. Previous studies have shown that increased S-100 levels in the CSF are an index of the active phase of cell injury in patients with acute multiple sclerosis exacerbations and in patients with intracranial tumors, acute encephalomyelitis, and spinal cord compression. Increased CSF levels of S-100 also have been demonstrated in patients with glioblastoma, hydrocephalus, subarachnoid hemorrhage, encephalitis, meningitis, Parkinson’s disease, cervical compression, polynuropathy, and cerebral infarction.

The GFA protein is the structural subunit of the astroglial filaments, which mainly are found in the fibrillary astrocytes. This protein is not readily soluble but is highly susceptible to degradation to watersoluble products. Previous studies have investigated GFA protein concentrations in CSF during acute
central nervous system damage as well as in chronic disorders. Increased GFA protein concentrations have been described as a consequence of acute encephalomyelitis, encephalitis, meningitis, intracranial tumors, cerebrovascular disease, syringomyelia, and dementia.\textsuperscript{7,12}

We initiated the present study to investigate changes in astroglial protein concentrations in the CSF during a 3-week period after cerebral infarction and to correlate these changes with other parameters. We determined the size of the infarction by computed tomographic (CT) scan and measured the blood–brain barrier dysfunction using the albumin CSF/serum ratio. We evaluated the clinical state of the patients using a simplified activities of daily living test. Furthermore, we measured myelin basic protein as an indicator of myelin breakdown. No previous systematic study of these astroglial CSF markers during ischemic stroke has been published, and the findings of the present study may be a valuable contribution to this area of research.

Subjects and Methods

We investigated 28 patients, aged 36–79 years (mean, 62 years; 19 men, nine women), with cerebral infarctions who were admitted to the Neurological Clinic of the University Hospital of Linköping. Examinations were performed 12–48 hours after onset of symptoms and again at 7 and 18–21 days after admission. The examination included clinical investigation, CT scan, and lumbar puncture. We excluded patients with signs of bleeding or severe cerebral edema on the initial CT scan. Ten patients did not undergo parts of the second and third examinations; one patient with a left internal carotid occlusion died of pulmonary embolism; two patients were transferred to another hospital; and in two patients, lumbar puncture could not be performed because of heparin treatment. In one other patient, the lumbar puncture was unsuccessful, and in four cases, CT scans were not feasible because of movement artifacts and an allergic reaction. Of these 10 patients, eight had small infarcts, and the remaining two had medium or large infarcts.

The clinical state of the patients was evaluated by a simplified activities of daily living test according to Katz et al\textsuperscript{13} and was graded as independent, slightly dependent, and totally dependent. Infarct size was described as follows: small (<1 cm), medium (1–5 cm), and large (>5 cm). A CT scan with matrix 160x160 was used. Control CSF was collected from 18 healthy patients (age range, 25–85 years) seeking medical advice for headache and found to be devoid of clinical signs indicating neurological disorder. Lumbar puncture was performed with the patient lying in a horizontal position. A standardized portion of 20 ml CSF was taken and thoroughly mixed before freezing at −20°C.

Albumin in serum and in CSF was determined by a nephelometric analysis of the antigen–antibody complex in a continuous-flow system. The CSF/serum albumin ratio was calculated.\textsuperscript{14} The S-100 protein CSF concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) described in detail elsewhere.\textsuperscript{15} The GFA protein concentrations also were measured with an ELISA as previously described\textsuperscript{16} but modified to increase the sensitivity. The protocol for this assay closely follows the procedure for the S-100 analysis,\textsuperscript{13} except that calcium chloride was exchanged for EDTA in the incubation buffer. The anti-GFA protein serum was used in a dilution of 1:5,000. The myelin basic protein CSF concentrations were determined with a radioimmunoassay method according to Karlsson and Alling.\textsuperscript{17}

The nonparametric Fisher's permutation test\textsuperscript{18} was used to test the differences observed between refer- ence and patient CSF S-100 or GFA proteins. The correlations between the different parameters of the patients at the various sampling times were tested using Spearman's rank correlation test.\textsuperscript{18}

Results

S-100 protein concentrations varied among control patients between 120 and 656 pM (mean±SD, 318.6±162.7). Control values for GFA protein concentrations varied between 83 and 231 pM (148.5±42.1). No sex-dependent (men=8, women=10) correlations were observed for either of the proteins. Generally, the 28 patients with cerebral infarction had significantly higher S-100 protein concentrations in the CSF during the first 48 hours (485.8±295.2) and at 7 days (457.4±225.7) after admission when compared with the control group (Figure 1). At 18–21 days, the S-100 concentrations did not differ from controls (292.6±102.5). GFA protein concentrations in the CSF during the first 48 hours (320.7±347.3) were significantly increased when compared with the control group (Figure 1). At 7 days, GFA protein concentration values also were increased (294.9±314.4), but this increment fell short of statistical significance (p=0.06). At 3 weeks, GFA protein
concentrations were normalized (145.2±36.4). S-100 and GFA protein concentrations of patients with medium-sized and large infarcts, as well as totally dependent patients, were significantly increased at the first sampling when compared to controls (Figures 2 and 3). The results of the myelin basic protein determinations will be presented in detail elsewhere (Zbornikova et al, unpublished observations). Briefly, high myelin basic protein concentrations were found after 1 week, whereas concentrations within 48 hours after admission and after 3 weeks were low.

Table 1 gives an overview of infarct size and localization. The initial clinical examination classified 13 patients as independent, four as slightly dependent, and 11 as totally dependent.

Table 1. Size and Localization of Infarctions in Studied Patients

<table>
<thead>
<tr>
<th></th>
<th>Central</th>
<th>Mixed</th>
<th>Cortical</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Large</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>19</td>
<td>7</td>
<td>2</td>
<td>28</td>
</tr>
</tbody>
</table>

Significant correlations were found between the CSF S-100 concentrations and infarct size, the clinical classification, the CSF GFA protein concentrations, and myelin basic protein concentrations at the first and second sampling (Table 2). In a similar way, GFA protein concentrations correlated significantly with infarct size, the clinical classification, and myelin basic protein concentrations at the first and second sampling (Table 2). Figure 4 displays S-100 and GFA protein concentration values plotted against each other at the time of the initial sampling. No significant correlations were observed at day 21. The CSF GFA but not the S-100 protein levels of patients with small infarcts were significantly correlated with clinical classification at the first and second sampling (p<0.05).

Discussion

This study shows that astroglial protein levels in the CSF increase early during a cerebral infarction. We observed significant elevations of S-100 and GFA protein concentrations within the first 48 hours of the disease; however, most of this increase occurred in patients with medium- or large-sized infarcts, with only a slight increase in patients with small infarcts.

The early leakage of astroglial proteins from their intracellular glial localization through the extracellular space into the CSF probably reflects the astroglial cell injury and death during the initial focal ischemic damage. Although this phenomenon is consistent with the low molecular weight and high solubility of S-100, the increase in CSF GFA protein is more difficult to understand. Because this protein is the subunit of the astroglial filament, it is not readily soluble. On the other hand, GFA protein is highly susceptible to degradation to water-soluble products by calcium-dependent proteinases. Ischemia in the brain causes an imbalance between extracellular and intracellular compartments, and one possible mechanism for cell death is the entrance of extracellular Ca++. It has been shown that postmortem degradation of GFA protein in human brain leads to the appearance of lower molecular weight GFA polypeptides and increased solubility of the protein in aqueous solutions. This degradation seems to be due to a calcium-dependent mechanism. Although this explanation seems credible, further research is needed to characterize the GFA protein in CSF after central nervous system damage. In any case, despite the different properties regarding solubility and susceptibility to degradation, as well as the differential localization of the two proteins in the protoplasmic...
TABLE 2. Spearman’s Rank Correlation Test Probability Values for Correlations at Different Samplings

<table>
<thead>
<tr>
<th></th>
<th>Albumin ratio</th>
<th>Clinical classification</th>
<th>Size</th>
<th>GFA protein</th>
<th>S-100 protein</th>
<th>MBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100 concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1–2</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>...</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Day 7</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td></td>
<td>...</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Day 18–21</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>GFA protein concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1–2</td>
<td>NS</td>
<td>&lt;0.0005</td>
<td>&lt;0.005</td>
<td>...</td>
<td>&lt;0.005</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Day 7</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>...</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Day 18–21</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>...</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

GFA, glial fibrillary acidic; MBP, myelin basic protein; NS, not significant.

and fibrillary astrocytes, an excellent correlation exists between CSF S-100 and GFA proteins within 48 hours after the infarction and after 7 days. Cerebrospinal fluid concentrations of S-100 and GFA proteins were still increased at 7 days, which might reflect the ongoing destruction of astroglial cells in the penumbra zone. Three weeks after patients were admitted, the astrogial protein levels in the CSF were normalized, suggesting a restoration of astroglial integrity. In accordance with previous results, the concentrations of the myelin basic protein in the CSF showed a different time course, with a major increment at 7 days after the ictus (Zbornikova et al, unpublished observations), indicating myelin breakdown. The increase in myelin basic protein correlates well with the elevations of S-100 and GFA proteins during the first week after the insult, but not after 3 weeks. A probable explanation of the diverse reactions of these markers in the CSF would be that myelin breakdown is a late event caused by the previous anoxic damage.

The infarct size markedly influenced the release of S-100 and GFA proteins during the first week of observation. The CSF levels of both proteins correlated well with the size of the ischemic area visualized on the CT scan (Table 2). Obviously, the leakage of astroglial proteins during the astrogial cell injury is proportional to the volume of the damaged tissue, and, consequently, a relation between CSF levels of the proteins and the clinical state of the patients also might be expected. Accordingly, the observed concentrations of astroglial proteins in CSF during the first 7 days after the ictus correlate with the clinical state of the patients (Table 2), as estimated by a simplified activities of daily living test. However, in this study, most of the patients were of the small-infarct type, and CSF levels of S-100 and GFA proteins among these patients did not differ significantly from the reference group (Figure 2). Thus, the relation between the astrogial protein levels and the clinical state of this subgroup of patients also was investigated. Even with this limitation, GFA protein concentrations correlated significantly with the results of the activities of daily living tests during the first week of disease, whereas S-100 concentrations did not. Consequently, GFA protein should be more suitable than S-100 as a marker of clinical outcome.

The present study did not include a long-term follow-up. Even so, we found significant correlations between the astrogial protein levels during the first week and the clinical state at 3 weeks (not shown), indicating a prognostic value of early determination of these parameters. In this context, it should be noted that the clinical state and outcome is a functional index of the central nervous system injury, which, among other factors, depends on the extent of the lesions as well as their localization. With this in mind, we investigated the relation of cerebral infarction localization with the astrogial protein concentrations and the clinical state of the patients. However, because there were few cortical strokes and because the large infarctions were predominantly of central or mixed type, we could draw no conclusions.

Other researchers have previously studied astrogial protein levels in CSF during ischemic stroke. In agreement with our study, concentrations of GFA protein and S-100 have been found to be increased shortly after cerebral infarctions. In the study of Hayakawa and coworkers, no correlation was ob-
served between the CSF GFA protein concentration and the clinical outcome of patients with ischemic stroke. In a study of 18 CSF samples from patients with infarctions,\(^5\) increased CSF S-100 concentrations were observed between 18 hours and 4 days after the ictus. It also was demonstrated that the S-100 levels were predominantly increased in patients with visible infarctions according to CT scans or brain scintigraphy. However, in this study, the correlation between the size of the ischemic area and the astroglial protein increment apparently was not investigated, and no correlation to the clinical outcome of these patients was found.\(^5\) Even so, in studies concerning other cerebral disorders, such as herpes encephalitis\(^22\) and subarachnoid hemorrhage,\(^23,24\) the S-100 concentration was found to be related to the clinical state, as well as to the outcome of the patient.

To summarize, during the first week after cerebral infarction, we found elevations of astroglial protein concentrations in CSF to be related to the size of brain damage and to the clinical state of the patients. Determinations of CSF S-100 and GFA protein concentrations therefore may be used as a diagnostic tool to estimate ischemic brain damage. We suggest that the use of these CSF markers would be of particular value in clinical trials of pharmacological agents such as calcium antagonists on stroke patients.

References
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