Levels in Stroke Patients of CSF Astroprotein, an Astrocyte-Specific Cerebroprotein

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SUMMARY Astroprotein (an astrocyte-specific cerebroprotein) levels in cerebrospinal fluid (CSF) were determined by radioimmunoassay in 47 stroke patients. (Astroprotein is immunologically identical to glial fibrillary acidic protein.)

Astroprotein levels in CSF increased markedly in acute cases of intracerebral hemorrhage and slightly to moderately in some acute cases of subarachnoid hemorrhage and cerebral infarction. In intracerebral hemorrhage, CSF astroprotein levels in the acute stage of the ictus reflected the size of the lesion and were used to estimate the clinical outcome. In subarachnoid hemorrhage and cerebral infarction, CSF astroprotein levels were related to the general neurological state.

Evidence obtained indicated that fundamentally different destructive and/or degenerative processes in the brain may be involved in intracerebral hemorrhage, subarachnoid hemorrhage and cerebral infarction and that determination of CSF astroprotein may have clinical significance in stroke patients.

Investigation of brain-specific proteins has advanced rapidly in recent years. Astroprotein designated by Mori is the major component of Tay-Sachs disease protein detected by Bogoch and is specific to astrocytes and astrocytoma cells. Glial fibrillary acidic protein (GFAP), extracted later by Eng et al., was immunologically proved to be identical with astroprotein.

It was reported that astroprotein levels in cerebrospinal fluid (CSF) increased in patients with glioma and it was suggested that the existence of a destructive process in the brain might cause the increase of astroprotein in CSF.

The purpose of the present paper is to analyze CSF astroprotein levels in stroke patients and study their significance.

Materials and Methods

Ninety-four samples of CSF were collected from 47 patients in acute or subacute stages of stroke; intracerebral hemorrhage (ICH) 22 patients, subarachnoid hemorrhage (SAH) 11 patients, and cerebral infarction (INF) 14 patients. Control CSF samples were obtained from 8 patients without clinical and laboratory evidence of intracranial diseases. From most of the patients (35) the first CSF samples were obtained by lumbar puncture in the acute stage within 4 days after the ictus without neurological procedures. From 14 patients, CSF samples were obtained on several occasions during the course of their diseases.

The CSF samples were stored at -60°C until assayed.

Methods of purification and radioimmunoassay of astroprotein have been described elsewhere and only a brief schematic outline is given in figs. 1 and 2. Current radioimmunoassay is able to detect astroprotein in the range of 5–1000 ng/ml.

Astroprotein concentration was assayed in all CSF samples and total protein level was simultaneously determined in 36 of the samples.

Results

Astroprotein concentrations in CSF of acute stage stroke patients are presented in fig. 3. The mean value of CSF astroprotein levels of the control patients was 10.0 ± 1.07 ng/ml as abnormal, the mean values of astroprotein levels in CSF obtained from acute stage stroke patients was 772.4 ± 90.14 ng/ml in ICH, 187.8 ± 109.05 ng/ml in SAH and 107.8 ± 57.77 ng/ml in INF. The astroprotein levels were markedly and constantly high in the ICH group. In some of the patients with SAH or INF, CSF astroprotein levels also increased but only slightly or moderately. CSF astroprotein levels of patients in acute stages of SAH or INF did not exceed 1000 ng/ml except for a case of SAH in critical condition. Statistical analysis by Student's t-test disclosed significant difference between the CSF astroprotein levels of ICH and SAH (p < 0.001), the levels of ICH and INF (p < 0.0005), and the levels of ICH and control (p < 0.0005).
glioma

0.05M phosphate buffer
homogenization
sonication
centrifugation

S-1
ammonium sulphate
30% saturated

S-2
affinity chromatography
c anti-AP (8M urea)
Sephadex G 25 gel filtration

AC
DEAE-sephadex A 25
column chromatography

FIGURE 1. Procedures for purification of astroprotein.

ICH

Figure 4 presents the relationship between CSF astroprotein levels in acute stage ICH and the size and location of hematoma with or without rupture into the ventricles. There is a tendency for patients with larger hematomas or a hematoma ruptured into the ventricles to show higher levels of astroprotein in CSF.

Figure 5 shows the changes of CSF astroprotein levels following the ictus when surgery is used. For a few days after the onset, the astroprotein levels were usually remarkably high, and then decreased gradually. The decrease of astroprotein in CSF was slower in patients treated by neurosurgical procedures, such as evacuation of hematoma, than in those treated conservatively.

SAH

Figure 6 presents the relationship between CSF astroprotein levels in patients with SAH and the

<table>
<thead>
<tr>
<th>131I-AP</th>
<th>100 μl</th>
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<tbody>
<tr>
<td>standard or sample</td>
<td>100 μl</td>
</tr>
<tr>
<td>anti-AP (X10,000)</td>
<td>100 μl</td>
</tr>
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1st incubation at 4°C for 48-72 hrs
1% NRS 100 μl
anti-R-G (X15) 100 μl

2nd incubation at 4°C for 20hrs
centrifuge
precipitate
count

FIGURE 2. Procedures for radioimmunoassay of astroprotein.

FIGURE 3. CSF astroprotein levels of acute stage stroke patients. ICH: intracerebral hemorrhage. SAH: subarachnoid hemorrhage. INF: cerebral infarction.

clinical grade by Hunt and Kosnik.11 The patients with higher grade showed higher levels of astroprotein in CSF.

INF

Figure 7 presents the relationship between CSF astroprotein levels and the levels of consciousness in the INF patients. No patients in this series was in coma at the time the first CSF specimen was collected. The patients with slighter consciousness disturbance showed lower levels of CSF astroprotein except for a patient with brain-stem infarct, in which CSF astroprotein on the 2nd day after ictus was very high. In this patient, CSF astroprotein became higher on the 3rd day and the infarct proved by CT scan to have become hemorrhagic.

Clinical Outcome

Figure 8 shows the relationship between CSF astroprotein levels in acute stroke and clinical outcomes. In ICH, the clinical outcome of the patients with lower astroprotein levels in CSF was better than those with higher astroprotein levels. No definite relationship was observed between CSF astroprotein levels and clinical outcome in the SAH and INF groups.
ICH CSF ASTROPROTEIN LEVELS IN STROKE/Hayakawa et al.

ICH

CSF ASTROPROTEIN (ng/ml)

10^4
10^2
10^1
10^0

Putaminal Thalamic Subcortical Cerebellar Brain stem

○ small hematoma with rupture into ventricle
● small hematoma without rupture into ventricle
□ large hematoma with rupture into ventricle
□ large hematoma without rupture into ventricle

FIGURE 4. The relationship between CSF astroprotein levels in acute stage ICH and the size and location of hematoma with or without rupture into the ventricles.

CSF Total Protein

Figure 9 presents the relationship between astroprotein and total protein levels in CSF. There was poor correlation between the 2 levels for all 3: ICH, SAH and INF.

Discussion

Astroprotein, which is immunologically identical to glial fibrillary acidic protein (GFAP), is the first protein found to be specific for astrocytes and is regarded as one of the components of the glial filament. Immunofluorescence studies using antisem to astroprotein demonstrated that the specific fluorescence was distributed in the cytoplasm of fibrillar astrocytes in the normal brain, reactive astrocytes and astrocytoma cells. Recently, this protein has been successfully purified and radioimmunoassay with double antibodies has been developed by Mori et al.

FIGURE 5. The changes of CSF astroprotein levels following the ictus of ICH with reference to surgical procedures.

SAH

FIGURE 6. The relationship between CSF astroprotein levels and the clinical grades in SAH patients.
Investigation of CSF astroprotein levels in patients with brain tumors indicated that CSF astroprotein increased in patients with gliomas, especially malignant gliomas.\(^9\)\(^{10}\) It seems likely that the mechanism of increased astroprotein in CSF might be connected to the existence and degree of degenerative and/or destructive processes in the brain.

The present study demonstrates that CSF astroprotein increases in acute stages of stroke, especially in ICH. In ICH, the fact that CSF astroprotein levels were higher in patients with larger hematoma would support the view that the increase of astroprotein in CSF may be concerned with destructive processes in the brain. In contrast to SAH and INF, the CSF astroprotein levels in ICH increased remarkably soon after the ictus. This may be due to differences of destructive mechanisms of brain tissue among the 3 types of stroke. ICH may cause more rapid and more serious destruction of brain tissue.

In SAH and INF, it was clear that the astroprotein levels in CSF were related to the patients' neurological state, i.e. clinical grade or consciousness level. The general neurological condition may be considered a reflection of the degree of destructive and/or degenerative processes in the brain.

There was poor correlation between CSF astroprotein levels and the prognosis in SAH and INF, though there was good correlation in ICH. The mechanism behind this difference remains obscure. In most of the SAH cases, intracranial aneurysms were found and treated neurosurgically. CSF was sampled within a few days of the onset of ictus. Possibly re-rupture of the aneurysms and late vasospasm, which happened after CSF sampling, affected the clinical outcome. Furthermore, clinical outcome may have been directly influenced by surgical procedures which followed. In the INF patients, the degenerative processes may be slow and fluctuating. The timing of CSF sampling should be further studied to clarify the relationship between CSF astroprotein levels and clinical outcome in SAH and INF.

There are numerous reports on changes in CSF i.e.
enzymes, electrolytes, etc. in various neuropathological conditions, but there have yet been no reports on changes in brain-specific protein levels in CSF of stroke patients. Figure 9 demonstrates that there was poor correlation between astroprotein and total protein levels in CSF. The result would indicate that the determination of astroprotein in CSF might have a different significance than total protein.

Evidence suggests that the measurement of astroprotein in CSF in the acute stage of stroke might have clinical usefulness.

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