S-100 protein and calmodulin levels in cerebrospinal fluid after subarachnoid hemorrhage

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The levels of two calcium-binding proteins, S-100 protein and calmodulin, were measured serially in the cerebrospinal fluid (CSF) of patients after subarachnoid hemorrhage (SAH) and aneurysm surgery. These two proteins have a similar molecular structure and are highly concentrated in the central nervous system (CNS). The levels of S-100 protein found in the earliest postoperative CSF samples correlated with the preoperative SAH grades. High S-100 protein levels in the CSF were found in patients with poor SAH grades. Moreover, the prognosis of the patients correlated with the S-100 protein levels in the CSF samples taken during the immediate postoperative period and with the daily changes of the S-100 protein levels. Severe diffuse cerebral vasospasm was followed by a sharp S-100 protein increase. These results suggest that S-100 protein levels in the CSF provide a useful index of organic damage in the CNS, and furthermore that S-100 protein levels and their changes may have prognostic value for patients after SAH. On the other hand, there was a lack of correlation between the calmodulin levels and the preoperative grade or outcome. It would be inappropriate, however, to speculate from the results of this study that these calcium-binding proteins in the CSF play any causative role in pathological processes such as cerebral vasospasm or brain ischemia after SAH, since changes in the levels of these proteins followed the onset of clinical signs of deterioration.

KEY WORDS • S-100 protein • calmodulin • subarachnoid hemorrhage • cerebrospinal fluid • aneurysm • vasospasm

The importance of the calcium ion as a general second messenger is well recognized in almost every biological field, not only in physiological but also in pathological states. Thus, in cerebrovascular diseases the calcium ion is thought to play an important role in various pathological cellular processes, such as brain ischemia, or cerebral arterial vasospasm after subarachnoid hemorrhage (SAH). The calcium ion itself is inactive, and its activity is mediated through a homologous class of calcium-binding proteins such as S-100 protein and calmodulin, which have a similar molecular structure and are highly concentrated in the central nervous system (CNS). In this study, we have measured the changes in S-100 protein and calmodulin levels in the cerebrospinal fluid (CSF) of patients with SAH due to rupture of an intracranial aneurysm. The protein levels were measured serially in CSF that was gathered via a cisternal drainage system placed after clipping the aneurysm. Correlation between the CSF levels of these calcium-binding proteins and the clinical course as well as possible implications in the pathophysiology of vasospasm were studied.

Clinical Material and Methods

Sixteen patients with ruptured intracranial aneurysms were selected for this study. They all underwent surgery within 3 days after their SAH. A CSF drainage system was placed in the basal cistern of these patients at the time of aneurysm surgery, left there for an average of approximately 2 weeks, and then either removed or replaced with internal shunts. The CSF samples were obtained daily, every morning, and preserved at −30°C until assayed. Control CSF samples were obtained from the lateral cerebellomedullary cistern at the time of microvascular decompression of hemifacial spasm in five patients who were otherwise healthy.

S-100 Protein Measurement

The S-100 protein levels were measured by the sandwich-type enzyme immunoassay method. Standard S-100 protein (10 to 1000 pg) in 0.1 ml solution or 0.1
ml of the CSF samples was incubated at 30°C for 6 hours with silicone rubber fragments (3 × 4 mm), coated with anti-S-100 antibody F(ab')2, in a final volume of 0.5 ml with a 0.01-M sodium phosphate buffer (pH 7.0) containing 0.3 M NaCl, 1 mM MgCl₂, 0.5% gelatin, 0.1% bovine serum albumin (fraction V), 0.1% NaN₃, and 1 mM calcium acetate. After the incubation, the reaction medium was removed by aspiration and the residual silicone rubber fragments were washed three times with 1 ml of a chilled 0.01-M sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl, 1 mM MgCl₂, 0.1% bovine serum albumin, and 0.1% NaN₃ (Buffer A). Then the silicone rubber fragments were incubated at 4°C overnight with 3 mU of the β-D-galactosidase-labeled anti-S-100 antibody Fab' fragments in 0.2 ml of Buffer A containing 1 mM calcium acetate. The pieces of silicone rubber were washed three times with Buffer A as before, and the β-D-galactosidase activity bound to the silicone rubber was assayed with 15 μmol of 4-methylumbelliferyl-β-D-galactoside as a substrate. The assay method is highly sensitive to a 0.1-ng/ml limit of detection.

Calmodulin Measurement

The levels of calmodulin were determined according to the ability of activation of calmodulin-deficient Ca²⁺-calmodulin-dependent cyclic nucleotide (guanine monophosphate: cGMP) phosphodiesterase that was purified from bovine brain, using purified calmodulin as a standard. The CSF samples were boiled for 2 minutes to inactivate the phosphodiesterase contained in the CSF, and centrifuged. Fifty microliters of the supernatant or 50 μl of the standard calmodulin (1 to 200 ng) was incubated at 30°C for 15 minutes with an incubation mixture of purified cGMP phosphodiesterase, 50 mM Tris/HCl (pH 8.0), 5 mM MgCl₂, and 4 μM ³H-labeled cGMP. The reaction was terminated by boiling the mixture for 5 minutes. Then 50 μg of snake venom was added and the mixture was again incubated for another 10 minutes. The fluid was poured into a small cation exchange resin column (AG 50W-X4, 200 to 400 mesh, 0.7 × 1.5 cm). After the column was washed with 15 ml of water, the product, ³H-labeled guanosine, was eluted with 1.5 ml of 3 N ammonium hydroxide. The amount of product was determined in a Beckman LS-233 liquid scintillation counter* with a 20-ng/ml limit of detection.

Results

S-100 Protein Concentrations

The mean concentration of S-100 protein in five control CSF samples was 0.96 ± 0.82 ng/ml (± standard deviation). Figure 1 left shows the relationship between the S-100 protein levels in patients during the acute phase (within 3 days) after SAH and the preoperative clinical grades following SAH, classified according to Hunt and Kosnik.10 Patients with more severe grades of SAH showed significantly higher S-100 protein levels (correlation coefficient r = 0.718, p < 0.013). The relationship of the changes in S-100 protein levels and the number of days after SAH are shown in Fig. 2 left.

There were clear differences in the S-100 protein levels versus the clinical outcome of the patients. In the good outcome group, the S-100 protein levels were lower than 5 ng/ml in the acute phase, and decreased rapidly to normal levels (below 1.5 ng/ml) within 1 week after the SAH. In the disabled group, the initial values were higher than 10 ng/ml in all but one case. The values slowly decreased, but remained higher than 2 ng/ml on the 7th day post-SAH. In the group of patients who died, the initial S-100 protein levels were also higher than those of the good outcome group, and two patients died of a severe cerebral vasospasm on the 8th and 11th days post-SAH. In these cases, when the protein levels showed a sharp rise, the patient’s condition deteriorated. Another patient died of disseminated intravascular coagulation on the 17th day post-SAH. In this case, the protein levels remained elevated after the 3rd day post-SAH, when the patient’s condition deteriorated, probably from vasospasm. When the changes in the S-100 protein levels were compared to the day of onset of vasospasm, it was obvious that the S-100 protein decreased toward the day of onset of vasospasm and then, after a time lag of about 1 day, increased (Fig. 3 left).

Calmodulin Concentrations

The mean value of calmodulin in the five control CSF samples was 26.5 ± 5.5 ng/ml. The relationship
CSF S-100 protein and calmodulin after SAH

Fig. 2. The postoperative time courses of the cerebrospinal fluid (CSF) protein levels in 16 patients. The patients were divided into three groups according to the final clinical outcome: "dead," "disabled," and "good." Left: There was an obvious correlation between the CSF S-100 protein levels and the clinical outcome — high levels in the "dead" and "disabled" groups and low levels in the "good" group. The circles indicate the day of onset of symptomatic vasospasm. The horizontal broken line indicates the S-100 protein levels in the control CSF samples (0.96 ± 0.82 ng/ml). Right: There was no correlation between the calmodulin levels and the clinical outcome. The horizontal broken line indicates the calmodulin levels in the control CSF samples (26.5 ± 5.5 ng/ml).

between the CSF calmodulin levels in the acute phase after SAH and the preoperative clinical grades following SAH is shown in Fig. 1 right. There was no correlation between these observations (r = 0.314, p < 0.84). In contrast to the correlation between the clinical course and the S-100 protein changes, no such tendency was seen in the CSF calmodulin levels (Fig. 2 right). Changes in the CSF calmodulin levels in relation to the onset of a vasospasm were not as obvious as those of the S-100 protein (Fig. 3 right).

Discussion

Both S-100 protein and calmodulin are calcium-binding proteins with a similar molecular structure called E-F bands. Although the S-100 protein was originally isolated from the CNS by Moore in 1965 and designated "S-100" because of its solubility in 100% ammonium sulfate, its biological role still remains unclear. The S-100 protein is a mixture of two major components, S-100a and S-100b with the subunit compositions αβ and ββ, respectively. The assay system that was employed in the present study is much more sensitive than any method previously used and is more specific for the S-100b protein. Although low levels of the S-100 protein were recently detected in various non-CNS tissues, this protein is considered to be one of the "nervous system specific proteins" densely localized in the glial and Schwann cells and can be utilized as an immunohistochemical marker for CNS tumors. Recently, S-100 protein levels have been examined by Michetti, et al., Sindic, et al., and Mokuno, et al., in the CSF of patients with various neurological disorders, such as meningocerebralitis, multiple sclerosis, Parkinson's disease, brain tumor, and cerebrovascular disease. Patients with acute extensive brain damage showed higher levels of the S-100 protein, and the authors mentioned above suggested that the S-100 protein levels in the CSF should be an index of active cell injury in the nervous system. In those studies, however, no attention was paid to the chronological changes of the S-100 protein levels in patients after SAH.

Calmodulin was originally discovered to be an activator of cyclic nucleotide phosphodiesterase by Cheung and Kakiuchi and Yamazaki in separate studies both conducted in 1970. It has been shown to activate many other enzymes, and now appears to be the chief mediator of Ca ++ effects in various cellular regulations. It is also considered to take part in "smooth-muscle contraction" through the regulation of myosin light-chain kinase. Calmodulin is widely distributed intracellularly in various organs: high levels are found in the brain, testis, and pituitary gland; intermediate levels in the lung, prostate, and adrenal gland; and low levels in the liver, kidney, and spleen. Significant amounts are contained in blood cells.

In this study, we measured serially the levels of the two calcium-binding proteins, S-100 protein and calmodulin, in the CSF of patients after surgery for a ruptured aneurysm. The S-100 protein levels in the earliest postoperative CSF samples correlated with the preoperative SAH grades. Moreover, the prognosis of the patients correlated with the levels of the S-100 protein in the CSF samples that were taken in the immediate postoperative phase of SAH, and with the changes in the S-100 protein levels during the 1st and 2nd postoperative weeks.
2nd weeks postoperatively. A poor prognosis was associated with high levels of S-100 protein (above 10 ng/ml) during the acute phase and the persistence of levels above 2 ng/ml for over 1 week. Severe extensive cerebral vasospasm was followed by a sharp rise in the S-100 protein levels. These results suggest that the S-100 protein levels in CSF are a useful index of organic damage to the CNS and that the S-100 protein levels and changes may have a prognostic value in patients after SAH. On the other hand, there was a lack of correlation between calmodulin levels and the preoperative grade or outcome. This is probably because large amounts of calmodulin exist not only in the nervous tissue but also in blood cells. Thus, the large amount of calmodulin released into the CSF after SAH may not reflect the damage in the nervous tissue.

It would be inappropriate to speculate from these results that CSF levels of the calcium-binding proteins, S-100 protein and calmodulin, play any causative roles in pathological processes, such as cerebral vasospasm or brain ischemia after SAH since changes in the levels of these proteins followed the onset of clinical signs of deterioration.

References


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