S-100 protein and neuron-specific enolase in CSF after experimental traumatic or focal ischemic brain damage

HANS-GÖRAN HÄRDEMARK, M.D., NILS ERICSSON, M.D., ZBIGNIEW KOTWICA, M.D., GERT RUNDSTROM, B.SC., IB MENDEL-HARTVIG, PH.D., YNGVE OLSSON, M.D., PH.D., SVEN PÄHLMAN, PH.D., AND LENNART PERSSON, M.D., PH.D.

Departments of Neurosurgery and Pathology, Uppsala University, Uppsala; Department of Neurology, Södersjukhuset, Karolinska Institute, Stockholm; and Department of Biochemistry and Immunology, Pharmacia AB, Uppsala, Sweden

Cerebrospinal fluid (CSF) markers of brain damage are potentially capable of providing quantitative information about the extent of certain neurological injury. The presence of such markers in CSF after brain damage is transient and it is essential to understand their kinetics if they are to be used in clinical practice. In the present study, the CSF concentrations of two neurospecific proteins, S-100 protein and neuron-specific enolase (NSE), were determined in rats before and repeatedly after one of two types of experimental brain damage: traumatic cortical injury and focal cerebral ischemia induced by middle cerebral artery (MCA) occlusion.

The two types of experimental brain damage resulted in significant differences in the kinetics of S-100 and NSE concentrations in CSF. Cortical contusion was followed by a rapid increase in both S-100 and NSE and a peak occurred in both after about 7½ hours, at which time the values declined toward normal. A second, smaller peak was seen after about 1½ days. The increase and decrease in S-100 and NSE levels in CSF was slower after MCA occlusion; a peak was seen after 2 to 4 days. Furthermore, S-100 was generally higher than NSE after trauma, whereas after MCA occlusion the NSE concentration was slightly higher than the S-100 value. These results support the use of CSF markers for estimation of the extent of brain damage in experimental models and forms a basis for the understanding of their kinetics, which is important for their use in clinical practice.

KEY WORDS • S-100 protein • neuron-specific enolase • ischemia • brain injury • rat

Cerebrospinal fluid (CSF) markers of brain damage have come to attract attention because they are potentially capable of providing quantitative information about the extent of certain types of neurological injury. They perhaps could be used for prediction of outcome and for therapeutic guidance. In the acute phase, clinical assessment of the extent of brain damage and prediction of the potential for restitution remain difficult. Basically, treatment of brain injury is instituted with the aim of limiting the extent of injury, in the hope that this will improve the outcome. Thus, the use of CSF markers for quantitative evaluation of treatment is logical because they may be assumed to reflect the extent of the injury rather than its clinical consequences.

Many substances are released into the CSF in conjunction with brain injury and are therefore of potential value as markers. An ideal marker should be exclusively intracellular and present in high concentration in the brain. Methods for quantifying S-100 protein and neuron-specific enolase (NSE) concentrations in CSF have been developed and applied in studies of patients with various neurological disorders involving brain damage as well as in experimental focal ischemia models. Both S-100 and NSE seem to meet the demands set out by Bakay, et al. They are intracellular proteins regarded as neuron-specific. Furthermore, NSE is present predominantly in neurons and S-100 in glial cells, so it is possible to obtain information derived from both types of cell.

The presence of biochemical markers in CSF after brain damage is transient, and it is essential to understand their kinetics if they are to be used in clinical practice. Many factors appear to influence the concentration of a marker in CSF. The nature and development of the pathological process are clearly im-
Experimental models could prove valuable for detailed studies of the kinetics of markers related to brain damage, because various types of standardized injury can be used in biochemical CSF studies. The aim of the present investigation was to compare the S-100 and NSE concentrations in rat CSF after two types of experimental brain damage: namely, traumatic cortical injury and focal cerebral ischemia induced by middle cerebral artery (MCA) occlusion.

Materials and Methods

This study involved 17 Sprague-Dawley rats (each weighing 375 to 480 gm), which were allowed food and water ad libitum. During all surgical procedures, the rats were anesthetized by intraperitoneal injection of 1.3 to 2 ml of a mixture containing 4.25% chloral hydrate and 0.97% pentobarbital, and were breathing spontaneously. After MCA occlusion, 20 ml of Ringer’s solution was injected subcutaneously.

Cannulation of the cisterna magna and sampling of the CSF were performed as described elsewhere. Briefly, a burr hole 2 mm in diameter was drilled in the midline directly over the external occipital crest; a No. 20 plastic catheter was inserted extradurally and pushed caudally along the inner surface of the occipital bone until the dura was perforated 1 to 2 mm above the foramen magnum. The catheter was then inserted directly into the cisterna magna. A total of 109 75- to 100-μl samples of CSF were gently aspirated via a connecting catheter. One to two samples were analyzed before the experimental brain lesion to ascertain the patency of the system and to ensure that the implantation of the catheter had not injured the brain. Sampling of CSF was carried out at various intervals for 3 to 7 days after the experimental procedure.

The CSF samples were centrifuged for 5 to 10 minutes at 5500 rpm. The supernatant was divided into two aliquots, one for NSE and one for S-100 measurement, and was frozen at −70°C. None of the supernatants showed hemolysis. The S-100 content was determined by enzyme-linked immunoassay as described by Rundström, et al. (unpublished data). Basically, this is a sandwich assay using two different monoclonal antibodies which detect the β subunit of S-100 protein. The lower detection limit was 1 ng of S-100/ml of CSF. The NSE content was determined by radioimmunoassay* as described previously. All determinations were performed under blind coding. The data were analyzed on a personal computer using commercial software (p < 0.05 was considered significant).

Cortical contusion was created in five rats by dropping a weight on the exposed parietal dura under controlled conditions. Focal cerebral ischemia was produced in eight rats by coagulation of the left MCA as described elsewhere. Sham operations were performed in three rats by exposing the parietal dura (one rat) but without dropping a weight, or by exposing the left MCA and slightly coagulating the adjacent cortex without touching the MCA. In one rat cisterna magna catheterization only was performed.

The rats subjected to cortical contusion were anesthetized and killed by decapitation. A coronal slice of the brain including the cortical contusion was rapidly cut and immersed in 2% 2,3,5-triphenyltetrazolium chloride at 37°C for 30 minutes to visualize the size of the contusion. The rats with MCA occlusion were anesthetized and perfused through the heart with 200 ml of 4% buffered formaldehyde. The brain was left in situ and immersed in the fixative overnight. Coronal sections of the brain were cut, embedded in paraffin, and stained with hematoxylin and eosin and van Gieson’s stains. All sections were examined by investigators without prior knowledge of the experimental procedure involved.

Results

Based on 15 CSF samples, CSF obtained before the experimental procedure or sham operation and CSF from the control rats contained an average of 3.0 ng S-100/ml and less than 4.0 ng NSE/ml. These values are in accordance with those found in an earlier study and those found by Rundström, et al. (unpublished data). Sham-operated rats showed a slight increase in concentrations of both S-100 and NSE, but much less than levels in rats subjected to trauma or MCA occlusion (Fig. 1).

Experimental Cortical Contusion

The traumatic cortical injury produced a sharply delineated contusion of similar size in all rats. A rapid increase in both S-100 and NSE concentrations in the CSF following the same temporal pattern occurred after the trauma. Distinct peak concentrations of both S-100

* Radioimmunoassay supplies obtained from Pharmacia AB, Uppsala, Sweden.
and NSE occurred after about 7±89 hours, after which the values rapidly declined toward normal. A second, smaller peak was seen after about 1±89 days (Fig. 2). The early S-100 concentrations were as a rule higher than the NSE concentrations. The mean of the highest S-100 value from each rat was 108.4 ng/ml, and the mean of the highest NSE value from each rat was 54.0 ng/ml. This difference was statistically significant (p < 0.05, Mann Whitney U-test).

Focal Cerebral Ischemia

In the rats with MCA occlusion, histological examination showed cerebral infarcts confined to the frontoparietal cortex and the lateral part of the caudoputamen. Both S-100 and NSE concentrations in the CSF increased following MCA occlusion, and essentially both markers showed the same temporal pattern in each rat. Small variations in S-100/NSE concentration were seen when comparing individual rats (Fig. 3). A peak concentration of both S-100 and NSE occurred after about 2 to 4 days, after which the values declined toward normal. The NSE concentration was as a rule slightly higher than the S-100 level. The mean of the highest NSE values from each rat was 30.8 ng/ml, compared with 24.2 ng/ml for S-100 values. This difference is not statistically significant. The levels of the markers in individual rats correlated with the histological findings; that is, the highest CSF concentrations were seen in the rats with the largest infarcts.

Discussion

In order to be able to make practical use of CSF markers of brain damage, it is clearly important to understand the kinetics of their transient appearance in CSF after various types of injury (such as traumatic or vascular), so that CSF samples can be taken at appropriate times. A single sample obtained at the right moment could yield important information, but a more detailed picture of the pathophysiological process will become evident if several samples are taken during the critical period while brain damage is developing.2,8

Experimental cortical contusion caused a rapid increase in the CSF concentration of both markers, the highest concentrations being noted after about 7±89 hours. This corresponds to clinical findings. In head-injured patients, the highest CSF concentrations of creatine kinase-BB,14,20 NSE, and S-100 were found within about 24 hours of injury. Brain contusion causes instant mechanical rupture of nerve cells, nerve fibers, and glial cells.1,6 The early concentration peak of the markers probably reflects this mechanical disruption of the tissue. Large amounts of cytoplasmic proteins such as S-100 and NSE may escape to the extracellular space, producing a "wave" of proteins reaching the CSF shortly after injury. Brain contusion also involves secondary ischemia damage due to impaired blood flow in the contused tissue. In this context, it is noticeable that a delayed and smaller peak concentration appeared after about 1±89 days, possibly reflecting such secondary damage.

Occlusion of the MCA causes focal cerebral ischemia in the ipsilateral frontoparietal cortex and lateral part of the caudoputamen;26 in rats allowed to survive, cerebral infarcts closely resembling those seen in man will develop.1,6 The NSE concentration in CSF increases after experimental focal cerebral ischemia,9,24 and the release of NSE from damaged cerebral tissue into the CSF reflects the development and size of the infarcts.9 The present study confirms these results and demonstrates that S-100 values in the CSF also mirror the
extent of the ischemic lesion. The peak concentration of both S-100 and NSE occurred later after MCA occlusion than after experimental cortical contusion. A possible explanation for this difference could be that the cellular injury resulting from focal cerebral ischemia is a more gradual process than that following trauma, and nerve cells may continue to succumb for at least several days after the production of ischemia. The present study thus demonstrates significant differences in the kinetics of CSF S-100 and NSE concentrations after trauma and focal ischemia. Furthermore, after trauma the S-100 value was as a rule higher than the NSE level, whereas after MCA occlusion the NSE concentration was slightly higher than the S-100 concentration. The reason for this is obscure, but it would seem that the use of several biochemical markers could yield additional information about the pathophysiology of various types of brain injury. Trauma causes mechanical disruption of the tissue, and both nerve cells and glial cells are damaged; during ischemia, on the other hand, the nerve cells may be more vulnerable than the glial cells. The contusion was localized to the cortex, whereas the cerebral infarct affected both the cortex and the lateral part of the caudoputamen. At present it is not known whether these structures differ with regard to the number of S-100- and NSE-containing cells, or whether cells in different regions of the brain contain varying amounts of these proteins. A conspicuous finding was that the relatively small cortical contusion resulted in such high S-100 and NSE concentrations in contrast to the levels noted after MCA occlusion, which apparently caused larger lesions. The same discrepancy has been observed in patients. The reason is unclear, but could be explained by the different pathophysiological processes involved. Trauma results in instant disruption of cells from which large amounts of intracellular proteins may escape into the CSF within a brief time, possibly before degradation and necrosis take place. The ischemic process, on the other hand, is apparently slower and involves coagulation and degradation of tissue proteins, probably including the marker proteins, so that an unknown amount of marker proteins will probably never reach the CSF. The degree of blood flow in the injured tissue may also influence the amount of marker protein that ultimately reaches the CSF. Transport of proteins from injured brain tissue into the CSF can be delayed or even inhibited by lowering the blood pressure, which suggests that blood flow does indeed play a role in the extracellular flow of water and constituents to the CSF. Conceivably, smaller quantities of marker might reach the CSF after focal ischemia in which the blood flow is severely impaired.

Acknowledgments

We thank Ms. Margareta Björing, Ms. Ulla Carlsson, Ms. Ingegärd Hjerrettson, Ms. Maud Salomonsson, and Ms. Karin Wettervik for excellent technical assistance.
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Manuscript received December 19, 1988.
This work was supported by the Swedish Medical Research Council; the Swedish Natural Science Research Council; the Laerdal Foundation for Acute Medicine; HKH Kronprinsessan Lovisas Förening för Barnsjukvård; Hans von Kantzows, Åke Wibergs, Ollie och Elof Ericssons, Tore Nilssons, and Syskones Svenssons Stiftelser, the Swedish Society of Medicine; and Pharmacia AB.

Dr. Kotwica (Department of Neurosurgery, Medical Academy of Lodz, Poland) was the holder of a Swedish Institute Research Scholarship.

This paper was presented in part at the 7th International Symposium on Intracranial Pressure and Brain Injury, Ann Arbor, Michigan, June 19-23, 1988.

Address reprint requests to: Lennart Persson, M.D., Ph.D., Department of Neurosurgery, Akademiska sjukhuset, S-751 85 Uppsala, Sweden.