Release of Superoxide Dismutase Into Cerebrospinal Fluid as a Marker of Brain Lesion in Acute Cerebral Infarction

Tage Strand, MD, and Stefan L. Marklund, MD

Background and Purpose: Evaluation of biochemical patterns in cerebrospinal fluid may add diagnostic and prognostic information. We tested to determine whether the concentration of superoxide dismutase in cerebrospinal fluid is a marker of brain tissue damage in acute ischemic stroke.

Methods: We investigated 36 acute ischemic stroke patients for cerebrospinal fluid activity of the enzyme superoxide dismutase on two occasions shortly after symptom onset (average, day 1 and day 4).

Results: In 75% of the patients, the first of two lumbar punctures revealed the maximal superoxide dismutase value. The amount in the cerebrospinal fluid was significantly correlated with the size of infarction on computed tomographic scan (p<0.001 by analysis of variance) and to functional impairment and stroke-related mortality during initial hospital stay (p<0.002). The correlation of initial superoxide dismutase concentration with the need for long-term institutional care and mortality at 3 months after the stroke was also significant (p<0.03).

Conclusions: We conclude that superoxide dismutase in cerebrospinal fluid is a marker of an acute brain lesion and has some value as a prognostic predictor. This small enzyme leaks rapidly from ischemically injured cells. (Stroke 1992;23:515-518)

KEY WORDS • cerebral infarction • cerebrospinal fluid • superoxide dismutase

Quantitative analysis of the biochemical constituents of cerebrospinal fluid (CSF) offers a method for evaluating various pathological alterations in the central nervous system. The blood–brain barrier reduces the migration of possible markers of central nervous system injury into systemic blood, although these same markers are easily released into the CSF, which is in dynamic equilibrium with the extracellular fluid of the brain. Several biochemical markers of brain damage in acute cerebrovascular disease have been reported.1

Superoxide dismutases (SODs) catalyze the dismutation of the superoxide anion radical \( 2 \cdot \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \) and have hitherto not been evaluated as markers of brain tissue damage. The SOD activity can be measured by three isoenzymes, the cytosolic 32-kd SOD (CuZnSOD),2 the mitochondrial 85-kd SOD (MnSOD),3 and the secretory 135-kd extracellular SOD (EC-SOD).4

Our aim in this study is to explore whether the concentration of SOD in the CSF is a marker of brain tissue damage in acute cerebrovascular disease and to correlate the CSF SOD concentration with the patients' clinical outcome.

Subjects and Methods

Cerebrospinal fluid samples were collected from 36 consecutive acute stroke patients of mean age 72.6±12.5 (range 30–90) years (52% males) at two early occasions (mean day 1 and day 4) after symptom onset. The patients were treated in the stroke unit at the Department of Internal Medicine, Umeå University Hospital, Umeå, Sweden. The routines and admission criteria to the unit are described elsewhere.5 Lumbar puncture samples macroscopically contaminated with blood were rejected. The CSF samples were stored at −80°C until the SOD assay was performed.

Computed tomographic (CT) scans (EMI Mark 1 head scanner) were performed as soon as possible after hospital admission (mean days 1–2 after symptom onset) and repeated after 1 week if the initial scan was inconclusive. The size of infarction was classified as follows: 0, no visible area of low attenuation; 1 (small), area of low attenuation corresponding to less than one third of the involved vascular territory supplied by the anterior, middle, or posterior cerebral arteries; 2 (large), area of low attenuation corresponding to more than one third of the involved vascular territory. In four patients who were unable to cooperate, CT scans were either inconclusive or not performed.

According to the International Classification of Diseases, 9th Revision, diagnostic criteria for classification of cerebrovascular diseases were as follows: nonembolic cerebral infarction, focal neurological deficits persisting more than 24 hours with no signs of hematoma on CT scan or at autopsy; embolic cerebral infarction, as above but with a potential source of cardiogenic embolus and...
a sudden onset of symptoms; transient ischemic attack, focal neurological deficit of presumed vascular origin with a duration less than 24 hours and without infarction visualized on CT scan.

Neurological examinations, including an assessment of activities of daily living (ADL) capacity, were performed on admission to the stroke unit, repeatedly during the acute phase, and also at a 3-month follow-up. The severity of neurological deficits was determined at the time of discharge from the stroke unit for the purpose of this study and at a 3-month follow-up. The severity was classified into three groups: I, no deficits or only minor deficits not affecting ADL capacity; II, moderate deficits only slightly affecting the ADL capacity; and III, severe deficits affecting or all or the majority of the ADL functions, including patients who died of stroke or stroke-related complications.

The analyses of SOD were performed with the direct spectrophotometric method employing KO$_2$ as previously described by Marklund, with some modifications. The data are presented as units per milliliter of cerebrospinal fluid. The method is about 40 times more sensitive than the commonly used xanthine oxidase–cytochrome C assay. One unit corresponds to 8.3 ng human CuZnSOD, 8.6 ng human EC-SOD, and 65 ng MnSOD. To distinguish between the cyanide-resistant MnSOD and the cyanide-sensitive isoenzymes CuZnSOD and EC-SOD, 3 mM cyanide was used.

We used CSF from 20 subjects of mean age 69 (range 60–74 years) as controls. Those subjects were included in an investigation of various CSF and serum parameters in healthy individuals. In the 20 healthy control subjects, the CSF SOD activity was 35.1±6.2 (mean±SD) units/ml. The SOD activity was inhibited 99.7% by cyanide, showing that the content of the cyanide-resistant isoenzyme MnSOD was negligible. Of the total activity, an average of 25% was adsorbed by immobilized anti-EC-SOD antibodies and the rest by immobilized anti-CuZnSOD antibodies. The major part (75%) of the SOD activity was thus given by CuZnSOD. That fraction is likely to increase in cerebral insults because of leakage of cytosolic CuZnSOD. For simplicity, we chose to study total CSF SOD activity in the patient groups. The data from our elderly controls are almost identical to those previously found in younger healthy persons (mean age 12 years).

For testing group differences in means, the $t$ test and one-way analysis of variance (ANOVA) were used according to the STATWORKS statistical package for microcomputers (Macintosh).

### Results

In 75% of the patients, the maximal SOD concentration in CSF (SODmax) was found at the first lumbar puncture as shown in Figure 1. In Figure 2, SODmax values are shown by diagnostic category. The patients with transient ischemic attacks ($n=4$) showed the lowest mean SODmax (35.0 units/ml), which did not differ from controls. Patients with nonembolic and embolic cerebral infarctions showed the highest mean SODmax (mean 71.9 and 57.1 units/ml, respectively). The difference between the cerebral infarction groups and the transient ischemic attack group was statistically significant ($p<0.026$ by ANOVA).

There was a significant correlation between the severity of neurological deficits on admission to the stroke unit and peak CSF SOD concentration ($p<0.002$) (data not shown). Figure 3 shows the patients grouped according to functional capacity at the time of discharge from the stroke unit and mortality during initial hospital stay. Patients with no or only minor functional impairments had mean SODmax 49.5 units/ml compared with 52.1 and 80.8 units/ml in the patients with moderate and severe disability (including deceased patients), respectively. The mean SOD values were significantly correlated to the degree of functional impairment ($p<0.002$ by ANOVA). Length of stay in the stroke unit was approximately 15 days. One patient with minor residual symptoms at discharge and without a visible lesion on CT scan showed a considerably elevated CSF SODmax value of 106.5 units/ml. This patient had generalized grand mal epileptic seizures on admission and was unconscious.

In 19 patients with visible lesions on CT scan, mean SODmax±SD concentration was 62.4±19 units/ml compared with 45.5±23 units/ml in those patients with no visible lesion ($n=13$). This difference was statistically significant ($p<0.03$ by $t$ test). Figure 4 shows that mean SODmax increased significantly with the volume of infarction ($p<0.001$ by ANOVA).
largest infarction on CT scan (area of low attenuation corresponding to more than one half the vascular territory supplied by the median cerebral artery) showed the highest SODmax concentration (112 units/ml).

At follow-up 3 months after the stroke, the patients who were institutionalized or deceased showed higher mean SOD concentrations in CSF during the acute phase (Figure 5). The difference reached a statistical significance of \( p<0.03 \) by ANOVA. One patient who was institutionalized 3 months after the stroke showed a normal SOD concentration (27.8 units/ml) in CSF. This patient had severe arthritis in several joints, which was the main reason for institutional care.

Discussion
The mean CuZnSOD activity in CSF rose as a result of cerebral infarction. CuZnSOD is likely to have leaked from the cytosol of damaged cells. The CSF CuZnSOD activity was still low, approximately 50 units/ml compared with the brain tissue CuZnSOD activity of around 18,000 units/g wet wt. The CuZnSOD cannot have leaked from plasma, which contains only 3 units/ml. Patients with larger lesions shown on CT scan and more severe neurological dysfunctions showed significantly higher SOD values. The SOD levels were also correlated to the need for long-term hospitalization and to mortality. The correlation was most strongly related to the degree of neurological dysfunction immediately after onset. Thus, it seems that SOD is a quantitative marker of the amount of ischemically affected cells in the central nervous system in the acute stage but may not be a good marker of permanent loss of cell function. One patient with TIA showed a modestly increased SOD activity. Also, one patient with generalized epileptic seizures at symptom onset, but with only minor residual neurological deficits and no visible lesion on CT scan, showed a considerable elevation of CSF SOD concentration. The number of patients with TIA included in this study (\( n=4 \)) is too small to allow any conclusion regarding whether SOD leaks from ischemically or metabolically disturbed cells, although the disturbance in cell function may clinically be fully reversible.

Many investigators have correlated brain tissue damage to elevated concentrations of various substances in the CSF. Studies of stroke patients have demonstrated significant correlations between extent of brain lesions and elevated CSF levels of brain-specific proteins, such as myelin basic protein, neuron-specific enolase, and astrogliosis. Correlations with extent of stroke-induced brain lesion and elevated CSF concentrations are also reported for intracellular enzymes such as adenylate kinase, the brain tissue-specific creatine kinase isoenzyme, intracellular proteins such as the S-100 protein, and unspecific markers of hypoxic metabolism such as lactate.

When evaluating CSF markers of tissue damage, the time course of marker rise and fall is important because the lumbar puncture procedure is not frequently repeated. The optimal time for spinal fluid sampling is not clear. Büttner et al. reported that the maximal CSF concentration of adenylate kinase (molecular weight 21,000 d) in patients with cerebral infarction was found on the third day after the stroke. Kjekshus et al. reported peak levels of CSF creatine kinase in patients with global cerebral ischemia postcardiac arrest from 48
to 72 hours after the event. In an earlier study evaluating myelin basic protein, the peak levels of CSF seemed to be reached 3–5 days after symptom onset in patients with cerebral infarctions, a somewhat longer delay than that for the small intracellular enzymes. This seems reasonable because the maximal tissue degeneration and myelin breakdown takes some time to develop. Enzymes of low molecular weights may leak within hours from ischemically disturbed membranes, which may explain why SODmax was frequently found at the first lumbar puncture (mean day 1, 8–36 hours after symptom onset). We know very little about how long SOD is detectable in the CSF and the time course of elimination, which may be rapid. After intracisternal injection of CuZnSOD in guinea pigs, the clearance from the CSF displayed a half-time of approximately 75 minutes. Factors such as the anatomic localization of lesion influence the amount of SOD detectable in the CSF at a given time point. This may explain why some patients with obvious lesions did not show elevated SOD values. The SOD assay technique applied may also not be optimal because the assay measured the activity of both the intracellular CuZnSOD and extracellular CuZnSOD. It is the small intracellular CuZnSOD molecule (molecular weight 32,000 d) that is the marker of cell injury.

The fluctuating visibility of infarction on CT scan during the acute phase of stroke is a well-documented phenomenon, and the possibility of repeated CT examinations is limited. Small lesions localized in critical regions of the brain (i.e., the capsula interna) may produce dramatic neurological deficits comparable with large hemispheric infarctions. When evaluating groups of patients with acute stroke, neurological examination, combined with CT scan or other imaging techniques, has great value for prognostic predictions. However, if the purpose is to predict the prognosis in a particular patient, CSF markers of tissue damage may add valuable information about the quantitative and qualitative extension of the brain lesion. This feature should make objective measures of this or related factors of importance in large-scale clinical therapeutic trials.

We conclude that SOD in CSF is a quantitative marker of brain tissue damage in acute cerebrovascular disease and has some value as a prognostic predictor.

References


8. Bucht G: Clinical and etiological studies on dementia of Alzheimer type and multifactorial dementia. Uned University Medical Disser-tations 1983, New Series No 97, ISSN 0346–6612


23. Marklund SL, Heikskela H, Westermark T, Sanbaruori P: Superoxide dismutase isoenzymes in cerebral spinal fluid and plasma of patients with obvious lesions did not show elevated SOD values. The SOD assay technique applied may also not be optimal because the assay measured the activity of both the intracellular CuZnSOD and extracellular CuZnSOD. It is the small intracellular CuZnSOD molecule (molecular weight 32,000 d) that is the marker of cell injury.

The fluctuating visibility of infarction on CT scan during the acute phase of stroke is a well-documented phenomenon, and the possibility of repeated CT examinations is limited. Small lesions localized in critical regions of the brain (i.e., the capsula interna) may produce dramatic neurological deficits comparable with large hemispheric infarctions. When evaluating groups of patients with acute stroke, neurological examination, combined with CT scan or other imaging techniques, has great value for prognostic predictions. However, if the purpose is to predict the prognosis in a particular patient, CSF markers of tissue damage may add valuable information about the quantitative and qualitative extension of the brain lesion. This feature should make objective measures of this or related factors of importance in large-scale clinical therapeutic trials.

We conclude that SOD in CSF is a quantitative marker of brain tissue damage in acute cerebrovascular disease and has some value as a prognostic predictor.
Release of superoxide dismutase into cerebrospinal fluid as a marker of brain lesion in acute cerebral infarction.
T Strand and S L Marklund

Stroke. 1992;23:515-518
doi: 10.1161/01.STR.23.4.515

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/4/515

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/