Ischemic Edema in Stroke

A Parallel Study with Computed Tomography and Cerebrospinal Fluid Markers of Disturbed Brain Cell Metabolism

ANDREAS TERENT, M.D., GUNNAR RONQUIST, M.D., KJELL BERGSTROM, M.D., ROGER HÅLLGREN, M.D., AND HANS ÅBERG

SUMMARY Thirteen patients with stroke and one with TIA had repeated examinations with computed tomography (CT) of the head, examination of the cerebrospinal fluid (CSF) for adenylate kinase, glutathione, lactate, and albumin and clinical evaluations during the first fortnight after onset. In 9 patients with cerebral infarction edema shown on the CT scans was maximal on days 2-5, after which it diminished. In 2 patients with intracerebral hemorrhage the edema appeared early as a zone of low-attenuation around the high-attenuation area. Most patients with large lesions deteriorated clinically during development of the edema. In 3 patients the CT scans were inconclusive, probably because the lesion was too small. Adenylate kinase activity was present in all CSF samples during the period 6 hours-5 days, while glutathione was occasionally present in the CSF in 12 of the 14 patients. These findings are believed to indicate cell swelling and a leak in the plasma membrane. Based on these observations, it is suggested that initial ischemic edema is intracellular in patients with cerebral infarction, and that adenylate kinase in CSF is a sensitive marker for this type of edema.

THE DEVELOPMENT of ischemic edema following cerebrovascular lesions is a complex process which may be harmful not only because of brain swelling and herniation, but also through interference with the microcirculation, thus possibly increasing the zone of ischemia. Cerebral edema generally is defined as an absolute increase of the brain tissue water content and from animal experiments it has been found that ischemic edema is initially intracellular, having a morphological resemblance to the edema produced by cytotoxic agents.

Cerebral ischemia induces disturbed cell metabolism reflected by an almost immediate reduction of energy-rich phosphorous compounds. This reduction of energy charge is followed within hours by decreased intracellular potassium, and increased water and sodium content in the brain tissue. Later the blood-brain barrier (BBB) becomes more permeable to serum proteins, which may accumulate, together with water, in the extracellular space of the brain, constituting vasogenic edema.

Computed tomography (CT) makes it possible to visualize edema in the human brain in vivo. This provides the opportunity to relate development of edema to biochemical changes in CSF which reflect metabolic dysfunction of brain cells. Adenylate kinase is a predominantly cytoplasmic enzyme normally not found in the CSF, but which appears under conditions known to be associated with even slight changes of plasma membrane function. Glutathione is an intracellular tripeptide found in the CSF in patients with major neurological signs. Increased lactate levels in the CSF reflect enhanced anaerobic glycolysis within the central nervous system.

The aim of the present study was to investigate further edema formation in patients with acute cerebrovascular lesions during the first fortnight after the ictus, a period usually covering maximum development of edema. Repeated CT scans and CSF analyses of adenylate kinase, glutathione and lactate were done for this study.

Patients and Methods

Patients

This study included 14 patients, 4 women and 10 men. The mean age was 65.3 years (range 58-83). The criteria for entering the study was that the patient had experienced rapidly developing clinical signs of focal disturbance of cerebral function with no apparent underlying cause other than vascular. One patient fitted the definition of transient ischemic attacks (TIA), the others had completed stroke. Diagnoses were based on neurologic exam, CT scan of the brain, and spectrophotometric analyses of CSF. No treatment for cerebral edema was given. Three patients died and their lesions were documented by autopsy.

Clinical Methods

The patients were examined on 4 occasions by the same examiner. The first examination took place as soon as possible after admission and usually within 10 hours from the onset of symptoms. The second examination was done after 2-3 days, the third after 4-5 days and the fourth after 11-16 days. On each occasion a neurological examination was performed followed by CT scan and lumbar puncture. The neurological findings were scored ranging from 0 (death) to 100 (normal), according to a modification of the Matthew scheme. With this scoring system speech, motor strength and a performance disability scale are judged especially important and make up 71 points of a maximal score. Aphasia, for instance,
Computed Tomography

CT scan was performed before and after contrast injection using an EMI 1010 head scanner with a 13 mm collimator. The contrast medium, meglumine metrizoate (Isopaque cerebral), was given at a dose of 1 ml/kg body weight, starting the second scanning 5-10 minutes after the contrast injection. In evaluation of the CT findings, statistics on the 160 x 160 matrix values were obtained using the standard EMI software program. In the pre-contrast CT scans the following was performed: planimetric area measurement of the lesion, recording the mean attenuation in a 2 cm² circular area in the center of the lesion in comparable sections, registration of ventricular compression and/or mid-line shift. The net attenuation value of the lesion was obtained by subtracting the attenuation value of the corresponding area of the non-affected hemisphere. This procedure was done in order to avoid the influence of linear drift of the X-ray attenuation from time to time. The scale of attenuation ranged from -1000 to +1000 Hounsfield units (HU). The standard deviation of the mean value of water was 3 HU. The measuring error of the examiner was about 2 HU, as judged from repeated measurements in normal parts of the brain scans. Thus the following criteria were used for planimetric work: the mean attenuation value of the measured area should differ more than 5 HU from the one of the corresponding parts of the opposite brain half; the SD of the whole lesion area should not markedly exceed the SD of the 2 cm² central part. These criteria reduced the risk that normal brain tissue or other irrelevant structures were included. The distance between tomographic cuts was approximated to 10 mm when determining the lesion volume, not taking into account the small differences between individual parts of the slice (9-11 mm). The whole lesion volume was represented by the sum of lesions in different slices.

Biochemical Methods

CSF (about 8 ml) was removed on each occasion. The fluid was collected in 5 separate portions subjected to the following analyses:

- Hemoglobin and protoporphyrin derivatives were detected by screening the samples at 415 nm (Soret's band). If the absorbance exceeded 0.040 the sample was subjected to a further spectrophotometric analysis in the range 400-650 nm for identification of typical curve profiles, e.g., oxyhemoglobin and bilirubin.

- Lactate was determined in 0.2 ml CSF by following the enzymatic conversion of NADH to NAD⁺.

- Adenylate kinase was analyzed in 0.5 ml volumes. Sampling and handling of the CSF material, as well as the analytical procedure, have been described elsewhere. Normal values are below 0.015 U/l (the lower limit of detection). As significant amounts of blood may contribute to the total adenylate kinase activity, 2 samples with blood contamination were discarded.

- Glutathione. The total glutathione of CSF was analyzed in 0.2 ml samples following the method of Brehe and Burch for other biological systems, with the exception that standard curves were performed using known amounts of glutathione suspended in pooled control CSF samples. The normal value is less than 0.05 μmol/l. 12

- Albumin was determined by a nephelometric technique, the normal value less than 300 mg/l.

Student's t-test was used for statistical analysis.

Results

The 14 patients were categorized in 3 groups according to the type of lesion as revealed by CT. Hemorrhagic lesions were found in 3 patients, 2 of them were intracerebral hemorrhages, 1 a hemorrhagic infarct. Eight patients were diagnosed as having consistent non-hemorrhagic infarct since they developed purely hypo-dense areas. Three patients lacked relevant CT findings of cerebral lesions and were classified as negatives. The clinical findings permitted a further sub-division into 3 groups: TIA, minor stroke and major stroke (table I). All 3 patients with hemorrhagic lesions had a major stroke and all patients with a normal CT scan had minor strokes.

The clinical scores at the different times of examination are shown in figure 1a. The condition of the patients with hemorrhagic lesions changed the most as compared to the others at admission. Clinical deterioration was noted on the second-third day in 2

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**Table 1. Sub-division Into 3 Groups**

<table>
<thead>
<tr>
<th>CT finding</th>
<th>Clinical picture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIA</td>
</tr>
<tr>
<td>Hemorrhagic lesion</td>
<td>3</td>
</tr>
<tr>
<td>Non-hemorrhagic infarct</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
</tr>
</tbody>
</table>

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**Figure 1.** Clinical score (a), lesion volume (b), and central hyper-hypodensity (c) in the individual patients. Patients No. 1 and 3 had intracerebral hemorrhages; patient No. 2 had a cerebral infarct, which did not become hemorrhagic until the fourth examination; patients No. 4-11 had non-hemorrhagic infarcts and patients No. 12-14 had negative CT scans (N). A score of 100 means normal function. Filled areas represent the blood clot and striped areas the edema surrounding it in figs. 1 b, c. Unfilled areas represent non-hemorrhagic infarcts in figs. 1 b, c.
Fig. 1a. Time after onset of symptoms

Fig. 1b. Volume c.m^3

Fig. 1c. HU Hyper-attenuation

HU Hypo-attenuation
FIGURE 2. Cerebellar hemorrhage. The edema appears as a low-attenuation zone around the high-attenuation area.

patients with hemorrhage after which a steady improvement occurred. Patients having non-hemorrhagic infarcts had a great spread of score at admission. Most of those with low scores had further deterioration on the second day, while those in better clinical condition had shown some improvement. All patients showed improvement until the end of the second week, but some patients with low scores on day 2 improved by day 4. The 3 patients without relevant changes on the CT scans had the best outcome.

The CT appearance of the lesions is presented as the change in central attenuation values (fig. 1 c) and total volume (fig. 1 b). From the start the intracerebral hemorrhages had a high-attenuation area surrounded by a low-attenuation area not changing much later on (fig. 2). The hemorrhagic infarct was not macroscopically hemorrhagic based on CT examination until day 13 when the hemorrhagic character of the lesion was easily recognized (fig. 3d). In the 2 patients examined within 6 hours after ictus neither of the infarcts was visible, but when scanned between 6 and 10 hours they had hypo-attenuation. The infarcts varied greatly in size. A common feature was the rapid increase of lesion volume until day 2-3, a steady volume at day 4-5 and a decreased volume from day 11-16. On the last study by CT, the lesion was hardly visible in one patient (fig. 4d) and was invisible in 2 others. The hypo-attenuation increased until day 4-5, after which it appeared less pronounced. Concomitantly, the border of the lesion became sharply demarcated. Border contrast enhancement was seen on day 2-5 in 4 patients. The same pattern was seen in another 3 patients on day 11-16, while 4 exhibited irregular contrast enhancement (table 2).

As seen in table 3, adenylate kinase activity was found in one patient out of three examined within 6 hours from onset. This patient was admitted to the hospital with a distinct TIA the day before the stroke. During the interval between 6 hours to 5 days adenylate kinase was found in all specimens of CSF. The last sample, taken on day 11-16 showed lowered activity, but the decrease was not significant (p > 0.05, table 4). Glutathione levels above normal

- **TABLE 2. Positive CT Examinations, Mass Effect and Contrast Enhancement, in Relation to Total Number of CT Examinations in Each Interval in 14 Patients**

<table>
<thead>
<tr>
<th>Time after onset of symptoms</th>
<th>Mass effect</th>
<th>Contrast enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1.6</td>
<td>0/14</td>
<td>0/14 f</td>
</tr>
<tr>
<td>2-3</td>
<td>3/13 t</td>
<td>1/13 t</td>
</tr>
<tr>
<td>4-6</td>
<td>0/12</td>
<td>7/12 t</td>
</tr>
</tbody>
</table>

- Faint borderline enhancement.
- Three cases of faint borderline and 4 cases of bright patchy enhancement.

- **TABLE 3. CSF Samples with Significant Adenylate Kinase Activity (≥ 0.015 U/l), Increased Glutathione (≥ 0.05 μmol/l) and Increased Lactate (≥ 2.3 mmol/l) Concentrations in Relation to Total Number of Analyses. Fourteen Patients Presented.**

<table>
<thead>
<tr>
<th>Time after onset of symptoms</th>
<th>Adenylate kinase</th>
<th>Glutathione</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6 h</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
</tr>
<tr>
<td>6-24 h</td>
<td>11/11</td>
<td>4/9</td>
<td>6/12</td>
</tr>
<tr>
<td>2-3 d</td>
<td>13/13</td>
<td>13/13</td>
<td>8/11</td>
</tr>
<tr>
<td>4-6 d</td>
<td>13/13</td>
<td>12/12</td>
<td>3/8</td>
</tr>
<tr>
<td>11-16 d</td>
<td>8/11</td>
<td>3/8</td>
<td>1/3</td>
</tr>
</tbody>
</table>

- **TABLE 4. Adenylate Kinase Activity, (normal value < 0.015 U/l), Glutathione, (normal value < 0.05 μmol/l), Lactate (normal value < 2.3 mmol/l) and Albumin (normal value < 300 mg/l) Concentrations in CSF in All Patients. Results are expressed as mean values ± SEM (Range in brackets).**

<table>
<thead>
<tr>
<th>Time after onset of symptoms</th>
<th>Adenylate kinase</th>
<th>Glutathione</th>
<th>Lactate</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1.6</td>
<td>0.275 ± 0.201 ^a</td>
<td>0.113 ± 0.026 ^a</td>
<td>0.078 ± 0.021 ^a</td>
<td>0.056 ± 0.017 ^a</td>
</tr>
<tr>
<td>(&lt;0.015 - 2.871)</td>
<td>(0.023 - 0.332)</td>
<td>(0.023 - 0.289)</td>
<td>(&lt;0.015 - 0.205)</td>
<td></td>
</tr>
<tr>
<td>U/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.10 ± 0.04 ^a</td>
<td>0.03 ± 0.02 ^a</td>
<td>0.06 ± 0.02 ^a</td>
<td>0.16 ± 0.12 ^a</td>
</tr>
<tr>
<td>μmol/l</td>
<td>(0 - 0.3)</td>
<td>(0 - 0.15)</td>
<td>(0 - 0.2)</td>
<td>(0 - 1.0)</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.87 ± 0.12 ^b</td>
<td>2.32 ± 0.30 ^b</td>
<td>1.94 ± 0.15 ^b</td>
<td>2.05 ± 0.25 ^c</td>
</tr>
<tr>
<td>mmol/l</td>
<td>(1.30 - 2.31)</td>
<td>(1.06 - 0.60)</td>
<td>(1.27 - 3.47)</td>
<td>(1.38 - 3.34)</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.256 ± 0.016 ^a</td>
<td>0.277 ± 0.021 ^a</td>
<td>0.281 ± 0.022 ^a</td>
<td>0.290 ± 0.025 ^a</td>
</tr>
<tr>
<td>g/l</td>
<td>(0.165 - 0.341)</td>
<td>(0.186 - 0.444)</td>
<td>(0.148 - 0.437)</td>
<td>(0.145 - 0.370)</td>
</tr>
</tbody>
</table>

- N = 13
- N = 11
- N = 12
- N = 8
- N = 9

x) all 14 patients included.
FIGURE 3. The development of cerebral infarct edema with mid-line shift and ventricular compression on CT scans (pre-contrast scans a-d and post-contrast scans e-g) and elevated adenylate kinase activity in the CSF. On day 13 small high-density areas indicate hemorrhages in the infarct. Patchy contrast enhancement is also seen, indicating damage to the BBB. Abscissa denotes 13 days.

were present throughout the same period in 12 of 14 patients but in less than 50 percent of the control specimens analyzed (tables 3, 4). Lactate levels above the upper normal range were found in one half the specimens analyzed on day 2-3 and in 3 on day 11-16 (tables 3, 4). Albumin levels were not significantly increased (table 4). To examine the possible influence of the serum content of adenylate kinase, glutathione and lactate on the CSF levels, the corresponding serum values were determined in 6 patients on the day of lumbar puncture. No correlation between serum and CSF concentrations was found.

Discussion

A low-attenuation area, as seen in patients with cerebral infarction and in those with hemorrhagic lesions, may be caused by several pathological changes in the brain. The present observations as well as pathological studies indicate that increased tissue water content is the cause of the hypo-attenuation during the first week after the onset. Ventricular compression and midline shift found in many of our patients indicate local brain swelling on day 2-5, when the hypo-attenuation is also maximal. Conclusions about
the type of edema present cannot be drawn from these observations. Contrast enhancement may represent gross BBB changes linked to vasogenic edema as is the case early in hemorrhagic lesions. The mechanism causing contrast enhancement observed during the first days after onset in patients with infarction is obscure. The absence of increased CSF albumin suggests other mechanisms than gross BBB damage. Hyperperfusion is the most likely cause, since gross BBB damage in infarction does not appear until the second week as judged from parallel CT and radionuclide examinations. The early damage to the BBB as seen in some animal experimental models does not apply directly to human pathology.

Edema visualized by CT might well be caused by metabolic changes on the cellular level, the link being the unusually high dependence of the neurone on oxygen to maintain normal levels of ATP. ATP is the
most important determinant of the adenylate charge potential in the cell. A lowered adenylate charge potential may result in a decreased electrochemical potential, since it has been demonstrated in an in vitro experimental system that the Na\(^+\) - and K\(^+\)-dependent ATPase activity is depressed by a lowered intracellular ATP concentration. Another cause might be the inhibitory action on the transport ATPase system exerted by free radicals interacting with the phospholipid moiety of the ATPase complex. A lowered electrochemical potential of the plasma membrane, whatever the cause, results in increased influx of water. These complex metabolic changes, underlying cytotoxic edema, are difficult to study in vivo in humans. It has been claimed that the integrity of the plasma membrane can be changed when intracellular ATP is low, resulting in an increased leak of intracellular constituents, such as adenylate kinase and glutathione, into the extracellular fluid.

The edema in patients with cerebral infarction is probably intracellular during the first days, as indicated by the finding of adenylate kinase activity in the CSF of all patients, which reflects cell swelling and an increased leak of the plasma membrane. The presence of adenylate kinase in CSF was usual, while glutathione was more sporadically found. The reason for this discrepancy between presence of the enzyme and the tripeptide is not known. Damage to the BBB is probably the most important determinant of the adenylate charge potential.

Conclusions

1. The primary edema of ischemic brain tissue without bleeding is due to intracellular water accumulation and an increased leak through the plasma membrane of the cells.

2. The time relationship between the biochemical processes at the cellular level, represented by CSF adenylate kinase, glutathione and lactate levels, are closely related to the macroscopical evidence of edema as judged by CT.

3. Adenylate kinase activity seems to be the most sensitive marker of disturbed plasma membrane function.

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Lactoferrin, Lysozyme, and β2-Microglobulin in Cerebrospinal Fluid

Elevated Levels in Patients with Acute Cerebrovascular Lesions as Indices of Inflammation

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SUMMARY Serial determinations of β2-microglobulin, lactoferrin and lysozyme in CSF were performed in 14 patients with acute cerebrovascular lesions. Marked elevations were noted in patients with cerebral bleeding or hemorrhagic infarction. Patients with infarction without signs of bleeding or with cerebrovascular lesions undetectable by computed tomography also had an increase in these proteins. The increase in CSF of β2-microglobulin, lactoferrin and lysozyme could not be explained by a damaged blood-brain barrier but was believed to be a local product of the central nervous system. Peak levels of lactoferrin and lysozyme were noted on day 2–3 after onset of symptoms. Lactoferrin then declined while lysozyme remained elevated for another few days. β2-microglobulin gradually increased reaching peak levels on day 4–5 and remained elevated even 2 weeks after the onset of symptoms. We suggest that the increases of lactoferrin, lysozyme and β2-microglobulin reflect various inflammatory reactions mediated by granulocytes, macrophages and lymphocytes, respectively.

THE APPEARANCE after a cerebrovascular lesion of various myeloid or lymphoid cell types in the damaged brain areas and in the cerebrospinal fluid (CSF) indicates a local cerebral inflammatory reaction. A prominent feature of an inflammatory reaction is the extracellular release of lysosomal proteins such as lysozyme and lactoferrin from activated macrophages and neutrophils. Lactoferrin is located in both the primary and secondary neutrophil granules, whereas lactoferrin is found only in the secondary granules. In addition, lysozyme is found in monocytes and macrophages. β2-microglobulin, which appears on the surface of most cell types as part of the histocompatibility antigens, seems in certain situations to reflect lymphocyte activity. Various cell lines produce β2-microglobulin in vitro but lymphoid cell lines especially secrete appreciable quantities, elevated levels of this protein in various body com-

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