Glutamate and taurine are increased in ventricular cerebrospinal fluid of severely brain-injured patients

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Abstract

Glutamate contributes to secondary brain damage, resulting in cell swelling and brain edema. Under in vitro conditions, increased extracellular levels of the amino acid taurine reflect glutamate-induced osmotic cell swelling. In vivo, increases in cerebrospinal fluid (CSF) taurine could, therefore, unmask glutamate-mediated cytotoxic edema formation and possibly differentiate it from vasogenic edema. To test this hypothesis, ventricular CSF glutamate and taurine levels were measured in 28 severely brain-injured patients on days 1, 5, and 14 after trauma. Posttraumatic changes in CSF amino acids were investigated in regard to extent of tissue damage and alterations in brain edema as estimated by computerized tomography. On day 1, CSF glutamate and taurine levels were significantly increased in patients with subdural or epidural hematomas (8±/−0.8/71+/−12 microM), contusions (21+/−4.1/122+/−18 microM), and generalized brain edema (13+/−3.2/80+/−15 microM) compared to lumbar control CSF (1.3+/−0.1/12+/−1 microM; p < 0.001). CSF amino acids, however, did not reflect edema formation and resolution as estimated by computerized tomography. CSF taurine correlated positively with glutamate, eventually depicting glutamate-induced cell swelling. However, parallel neuronal release of taurine with its inhibitory function cannot be excluded. Thus, the sensitivity of taurine in unmasking cytotoxic edema formation is weakened by the inability in defining its origin and function under the conditions chosen in the present study. Overall, persisting pathologic ventricular CSF glutamate and taurine levels are highly suggestive of ongoing glial and neuronal impairment in humans following severe traumatic brain injury.
Glutamate and Taurine Are Increased in Ventricular Cerebrospinal Fluid of Severely Brain-Injured Patients

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ABSTRACT

Glutamate contributes to secondary brain damage, resulting in cell swelling and brain edema. Under in vitro conditions, increased extracellular levels of the amino acid taurine reflect glutamate-induced osmotic cell swelling. In vivo, increases in cerebrospinal fluid (CSF) taurine could, therefore, unmask glutamate-mediated cytotoxic edema formation and possibly differentiate it from vasogenic edema. To test this hypothesis, ventricular CSF glutamate and taurine levels were measured in 28 severely brain-injured patients on days 1, 5, and 14 after trauma. Posttraumatic changes in CSF amino acids were investigated in regard to extent of tissue damage and alterations in brain edema as estimated by computerized tomography. On day 1, CSF glutamate and taurine levels were significantly increased in patients with subdural or epidural hematomas (8 ± 0.8/71 ± 12 μM), contusions (21 ± 4.1/122 ± 18 μM), and generalized brain edema (13 ± 3.2/80 ± 15 μM) compared to lumbar control CSF (1.3 ± 0.1/12 ± 1 μM; p < 0.001). CSF amino acids, however, did not reflect edema formation and resolution as estimated by computerized tomography. CSF taurine correlated positively with glutamate, eventually depicting glutamate-induced cell swelling. However, parallel neuronal release of taurine with its inhibitory function cannot be excluded. Thus, the sensitivity of taurine in unmasking cytotoxic edema formation is weakened by the inability in defining its origin and function under the conditions chosen in the present study. Overall, persisting pathologic ventricular CSF glutamate and taurine levels are highly suggestive of ongoing glial and neuronal impairment in humans following severe traumatic brain injury.

Key words: brain edema; cell swelling; cerebrospinal fluid; contusion; glutamate; taurine; traumatic brain injury

INTRODUCTION

TRAUMATIC BRAIN INJURY is associated with structural, functional, and metabolic impairment of glial, neuronal and endothelial homeostasis (McIntosh et al., 1996). One of the consequences is an increase in extracellular glutamate due to sustained neuronal release, attenuated glial uptake and passive translocation from blood via a damaged blood-brain barrier (Katayama et al., 1990; Westergren et al., 1994). A close relationship between the degree of traumatic brain injury and the extent of extracellular glutamate accumulation has been demonstrated in animal (Palmer et al., 1993) and clinical studies (Bullock et al., 1995). The excitatory amino acid

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glutamate is known for its toxic potential and has been shown to cause gial and neuronal swelling and possibly cell death (Choi et al., 1987; Schneider et al., 1993). Intracellular accumulation of water referred to as cytotoxic brain edema is attributed to impaired ionic and energetic homeostasis (Katayama et al., 1990; Katayama et al., 1992; Nilsson et al., 1993; Nilsson et al., 1996) and is believed to develop sequentially or parallel to vasogenic edema formation. Glutamate-induced cell swelling is linked to compensatory release of the volume-regulatory amino acid taurine (Menéndez et al., 1989; Dutton et al., 1991; Saransaari and Oja, 1991), which could possibly reflect developing cytotoxic edema formation under in vivo conditions.

In brain-injured patients, posttraumatic recognition of secondary brain damage in terms of cytotoxic edema formation would allow to counteract and possibly prevent further cellular perturbation. In clinical routine, however, computerized tomography (CT) is only of limited value in unmasking glutamate-induced cell swelling as vasogenic and cytotoxic edema formation are not sufficiently discernible in pericontusional brain structures. Therefore, analysis of an endogenous substance of cellular origin, for example, taurine, could theoretically reveal otherwise occult cell swelling.

Previous studies have shown that analysis of cerebrospinal fluid (CSF) allows to estimate ongoing pathologic changes within cerebral and spinal tissues (Baker et al., 1993; Shimada et al., 1993; Palmer et al., 1994; Stover et al., 1997).

Therefore, the aims of the present study were to investigate changes in ventricular CSF glutamate and taurine levels of head-injured patients in regard to the extent of structural brain damage and to elucidate if taurine unmasks progression and resolution of brain edema as assessed by sequentially performed CT.

**MATERIALS AND METHODS**

**Study Population**

Studied patients with isolated brain injury were admitted with a GCS ≤ 8 to the University Hospital Zürich within 12 h after trauma. Following CT evaluation the patients were craniotomized to remove subdural or epidural hematomas and to implant intraventricular catheters. These indwelling catheters were inserted through nontraumatized brain areas. Patients with significant injuries to the thorax, abdomen, pelvis, or extremities were excluded. On the intensive care unit (ICU), the intubated and mechanically ventilated patients were treated according to a standard protocol (Stocker et al., 1995), which was not influenced by the present investigations. Parenteral nutrition with amino acid–containing solutions was not performed in these patients. All patients received enteral nutrition immediately upon arrival on the ICU. Neurological outcome of these patients was assessed 6 months after trauma. The study protocol was approved by the University Hospital Medical Ethics Board.

**Evaluation of Brain Damage According to Computerized Tomography**

CT scans were performed upon admission, followed by control scans on days 1, 5, and 14 after trauma. Extent of brain damage was assessed by calculating size of contusions and perifocal edema using the formula for ellipsoids [volume = \( \frac{4}{3} \pi \times a \times b \times c \) (cm\(^3\))]. Generalized edema was diagnosed by the absence or compression of cerebral sulci, ventricles, and basal cisterns (Weisberg et al., 1990).

**CSF Sampling and Control CSF**

CSF was collected on ice over 24 h daily until removal of catheters. Samples were centrifuged at 1,000 revolutions per minute (rpm) for 10 min at 4°C and frozen at −70°C until further analysis. Control CSF samples were taken from neurological patients undergoing diagnostic lumbar puncture. They were free of any pathologic findings as previously described in detail (Stover et al., 1997).

**Amino Acid Analysis**

Amino acids were analyzed by high-performance liquid chromatography (HPLC) (®Waters Millipore Corp., Milford, MA) using orthophthalaldehyde precolumn derivatization (Fürst et al., 1990). Deproteinized CSF samples were mixed with potassium carbonate, centrifuged, and stored at −70°C until analysis. Fluorescence detection was set at 330 nm (excitation) and 450 nm (emission wave length). Stationary phase was a Spherisorb C-18 column (125 × 4 mm; 3-μm particle size). Mobile phases were (a) stock buffer mixed with 1% tetrahydrofuran and 5% acetonitrile (pH 7) and (b) stock buffer-acetonitrile (50/50; pH 7.2). Stock buffer consisted of 1.724 g/L sodium dihydrogen phosphate and 1.77 g/L disodium hydrogen phosphate (40/60). After injection, a stepped gradient at a flow rate of 0.6 ml/min was applied (0–5 min 100% A, 5–52 min 0–95% B, 52–60 min 95–0% B). A mixture of amino acids was analyzed as external standards. Sample peak areas were compared to areas of corresponding standard amino acids of known concentration, which allowed calculation of measured sample concentration.

**Statistical Analysis**

Results are presented as mean ± standard error of the mean (SEM). Investigated parameters were compared for
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Table 1. Epidemiological Data of Investigated Patients According to the Extent of Structural Brain Damage

<table>
<thead>
<tr>
<th></th>
<th>SDH/EDH</th>
<th>Contusions</th>
<th>Generalized edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>4/1</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years</td>
<td>36 ± 6</td>
<td>35 ± 4</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>Range</td>
<td>20–46</td>
<td>16–60</td>
<td>17–67</td>
</tr>
<tr>
<td>Females/males</td>
<td>1/3</td>
<td>4/12</td>
<td>1/6</td>
</tr>
<tr>
<td>ICU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td>21 ± 5</td>
<td>33 ± 3</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>Range</td>
<td>12–31</td>
<td>16–48</td>
<td>9–36</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>—</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Vegetative state</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Severe disability</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Moderate disability</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Good recovery</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; SDH, subdural hematoma; EDH, epidural hematoma.

Significant differences within and between patients with different degrees of structural brain damage assessed by CT scans using one-way analysis of variances (ANOVA), Kruskal-Wallis, and Dunn’s tests or ranked-sum test for nonparametric data, where appropriate. Correlations of neurotransmitters were performed by linear regression analysis. Differences between the groups were rated significant at *p* < 0.05.

RESULTS

Patient Data

A total of 28 brain-injured patients were studied and grouped according to initial CT findings: five patients presented with subdural or epidural hematomas only, while 16 showed contusions, and seven had generalized brain edema. Age distribution was comparable in all groups, ranging from 16 to 67 years. The female/male ratio was similar in patients with subdural or epidural hematomas and contusions, whereas male patients presented more often with generalized brain edema. Hospitalization on the ICU ranged from 9 to 48 days and was shortest in patients with sole subdural or epidural hematomas. Neurological outcome for all patients is shown in Table 1.

Extent of Structural Brain Damage in Patients

Serial CT scans performed in patients with contusions revealed a significant increase in contusion size and perifocal edema during the first 24 h (Table 2). Thereafter, contusions and perifocal edema showed a tendency to resolve. Their calculated sizes, however, remained significantly increased by day 14 compared to the initial assessment. Performed CT studies in patients with subdural or epidural hematomas did not show any additional contusions or secondary ischemic damage adjacent to immediately removed hematomas. The degree of generalized brain edema resolved during the study period as gyri and sulci, and the basal cisterns became discernible. Quantification of generalized brain edema, however, was not done.

Influence of Brain Damage on CSF Amino Acids

CSF glutamate and taurine levels reflected the extent of traumatic brain damage (Table 3). They were significantly higher compared to control lumbar CSF values from nontraumatized patients (glutamate/taurine: 1.3 ± 0.1/12 ± 1 μM; *p* < 0.001). On day 1 after trauma, lowest values were found in patients with subdural or epidural hematomas (8 ± 0.8/71 ± 12 μM), while higher
controls vs. contusion, 


day 

corresponding to the fifth posttraumatic day (14 ± 1.6 μM; p < 0.001) followed by a significant decrease by day 14 (6 ± 1.3 μM; p < 0.001). CSF taurine, however, did not show similar changes. On day 1, CSF glutamate and taurine concentrations were significantly higher in patients with contusions compared to those with generalized brain edema (21 ± 4.1/122 ± 18 versus 13 ± 3.2/80 ± 15 μM; p < 0.05) and remained unchanged thereafter. CSF serine, an amino acid lacking any transmitter function within the central nervous system, remained remarkably stable in all brain-injured patients. Progression and resolution of brain damage as estimated by computerized tomography was not reflected by CSF amino acids despite a trend to smaller contusions and resolving perifocal and generalized brain edema by day 14.

**Mutual Dependency Between CSF Glutamate and Taurine Levels**

CSF taurine and glutamate concentrations of the three selected time points (days 1, 5, and 14 after trauma) were pooled for each group of investigated patients to perform regression analysis. Correlating CSF taurine with glutamate levels revealed a mutual dependency between these two amino acids in patients with contusions (taurine = 34.8 + 3.4 × glutamate; n = 48; r = 0.56; p < 0.001) (Fig. 1A) and generalized brain edema (taurine = −5.1 + 6.1 × glutamate; n = 21; r = 0.95; p < 0.001) (Fig. 1B). Patients with subdural or epidural hematomas alone did not reveal a significant correlation.

**FIG. 1.** (A) Significant correlation between CSF taurine and glutamate levels in patients with contusions (solid circle; n = 64; r = 0.56; p < 0.001). (B) Significant correlation between CSF taurine and glutamate levels in patients with generalized brain edema (open square; n = 32; r = 0.95; p < 0.001).
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DISCUSSION

Bed-side monitoring of pathological changes following traumatic brain injury in humans is facilitated by sampling of CSF via intraventricular catheters. In 28 severely brain-injured patients, ventricular CSF glutamate and taurine levels reflected the extent of structural brain damage as higher values were measured in patients with contusions and generalized brain edema, while low values were found in patients with subdural or epidural hematoma.

Sampling of ventricular CSF reflects neurochemical alterations within cerebral tissue as increased concentrations are moved passively via bulk flow towards the ventricles (Baker et al., 1993; Shimada et al., 1993; Palmer et al., 1994). Preserved glial and neuronal uptake mechanisms, and mixture with cerebrospinal and extracellular fluid are believed to dilute and reduce free amino acid levels measured in the ventricular system. Consequently, ventricular transmitter concentrations only represent a small fraction of the actual levels found in the vicinity of damaged tissue. Emerging experimental and clinical data underlines the importance of continuously monitoring the cerebral extracellular space of brain-injured patients which is best accomplished by microdialysis (Bullock et al., 1995; Zauner et al., 1996; Landolt and Langemann, 1996). Characterizing interstitial levels of amino acids with this elegant technique is superior to collecting CSF via indwelling ventricular catheters as microdialysis allows detection of minute, fast, and topographically localized changes which are masked by time-consuming diffusion processes, bulk flow and dilution of CSF within the ventricular system.

Under physiological conditions transmitters are maintained at low levels within the extracellular space due to neuronal and glial uptake (Danbolt, 1994). Normal extracellular glutamate and taurine levels are within the micromolar range while they are stored in millimolar concentrations intracellularly (Ottersen, 1988; Storm-Mathisen et al., 1992). Intact ionic and energetic homeostasis is indispensable in preventing uncontrolled leakage down existing concentration gradients as their transmitter function is not limited enzymatically within the synaptic cleft as seen for acetylcholine (Danbolt, 1994).

In traumatic and ischemic brain injury, uncontrollable release of glutamate (Nilsson et al., 1990; Hagberg et al., 1985) is attributed to general depolarization and impaired ionic homeostasis (Katayama et al., 1990; Katayama et al., 1992; Nilsson et al., 1993, 1996), inhibition of uptake by arachidonic acid and free oxygen radicals (Volterra et al., 1994), and reversal of the energy-dependent glutamate transporter due to ionic and energetic impairment (Madl and Burgesser, 1993). Primary brain injury is complicated by local ischemia known to occur in the presence of contusions and subdural hematoma (Bullock et al., 1991; Muizelaar and Schröder, 1994; Kochanek et al., 1995) despite successful maintenance of global cerebral perfusion pressure. Posttraumatic ischemic tissue damage, in turn, is associated with sustained release of glutamate (Bullock et al., 1991). This could possibly explain persisting pathologic CSF values in all patients and the secondary increase in CSF glutamate seen by the fifth postransfusion day in patients with subdural hematoma.

Observed progression of brain edema during the first 2 postransfusion days is in accordance with experimental findings (Eriskat et al., 1994; Kochanek et al., 1995). Enlargement of perifocal edema is related to evolving vasogenic and cytotoxic edema which seems to persist up to 14 days after trauma. The excitotoxin glutamate is widely accepted to contribute to these pathologic alterations. According to in vivo and in vitro studies glutamate damages the blood-brain barrier (Zuccarello and Andersen, 1993; Mayhan and Didion, 1996) and causes cellular swelling (van Harreveld and Fifkova, 1971; Schneider et al., 1993).

Artificial increases in CSF amino acid levels due to blood-brain barrier damage as seen in animal studies (Kempski et al., 1990; Koizumi et al., 1997) cannot be excluded in the present study. These artificial increases, however, seem subordinate since CSF serine levels remain rather stable in all investigated patients. Generalized edema formation is seen as the radiological correlate to cytotoxic edema (Weisberg et al., 1990) and thus should not be associated with a damaged blood-brain barrier. Therefore, pathologic CSF glutamate and taurine levels in patients with hemispheric edema underline the assumption that alterations in ventricular CSF transmitters reflect glial and neuronal impairment and are not increased due to passive translocation via a damaged blood-brain barrier.

Significantly increased ventricular CSF concentrations of the amino acid taurine and the positive correlation with glutamate in the investigated brain-injured patients suggest a mutual dependency between these two amino acids. Among other functions, taurine is known to regulate cell volume as it is released nonvesicularly. As taurine leaves swollen cells it shifts ions and water for stochiometric reasons from the intracellular to the extracellular compartment restoring initial cell volume (Pasantes-Morales et al., 1990). There is substantial evidence that glutamate induces osmotic cell swelling as ionic homeostasis is disturbed and Na+ accumulates intracellularly (Barbour et al., 1988; Kimelberg et al., 1989; Schneider et al., 1993). Glutamate-mediated increase in cell volume, in turn, has been shown to result in an increased release of taurine.
(Saransaari and Oja, 1991). Thus, the positive correlation between taurine and glutamate in the investigated patients could reflect underlying cytotoxic edema formation. Apart from osmoregulation, taurine is also known to exert neuromodulating (Kuriyama, 1980) and inhibitory functions (Okamoto et al., 1983; Taber et al., 1986). Neuronal excitation results in a sustained release of taurine (Magnusson et al., 1991) regulating exocytosis of excitatory amino acids with the aim of balancing glutamate-mediated excitotoxicity (Kamisaki et al., 1993). Co-release of these two amino acid transmitters is seen in the fact that changes in extracellular taurine levels occur in conjunction with alterations of free glutamate concentrations in traumatic and ischemic injuries (Nilsson et al., 1990; Hagberg et al., 1985) and has also been demonstrated in cultured neurons and astrocytes (Holopainen et al., 1989). Therefore, observed parallel increases in CSF taurine and glutamate levels in brain-injured patients do not allow to differentiate cytotoxic from physiologic effects, as they most likely occur at the same time. Consequently, analysis of taurine in ventricular CSF as collected over 24 h in brain-injured patients is only of subordinate value in possibly unmasking developing and resolving cytotoxic edema.

Progression and resolution of pericontusional and generalized brain edema as estimated by computerized tomography is not reflected by changes in rather stable CSF amino acids despite a trend to lower values by the end of the study period. Therefore, it is impossible to define if pathologic CSF values precede, follow or occur in conjunction with radiological findings. This is best explained by the multitude of interacting uptake and release mechanisms in damaged and surrounding tissue.

The present investigation revealed ventricular CSF glutamate and taurine levels to remain pathologically elevated up to and including day 14 after trauma in severely brain-injured patients. These findings are highly suggestive of persisting glial and neuronal impairment with a preserved risk of secondary brain damage in terms of neurotoxic cell damage lasting at least two weeks after the traumatic insult.

REFERENCES


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