Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans

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Evidence from models of traumatic brain injury implicates excitotoxicity as an integral process in the ultimate neuronal damage that follows. Concentrations of the excitatory amino acid glutamate were serially measured in the cerebrospinal fluid (CSF) of patients with traumatic brain injuries and in control patients for comparison. The purpose of the study was to determine whether glutamate concentrations were significantly elevated following traumatic brain injury and, if so, whether they were elevated in a time frame that would allow the use of antagonist therapy. Cerebrospinal fluid was sampled fresh from ventricular drains every 12 hours and analyzed using high-performance liquid chromatography for the excitatory amino acids. The peak concentrations of glutamate in the CSF of the 12 brain-injured patients ranged from 14 to 474 µM and were significantly higher than those in the three control patients, 4.9 to 17 µM (Mann-Whitney U-test, p < 0.02). Glutamate concentrations in five of the eight patients who were still being sampled on Day 3 were beyond the control group range. The implication of this study is that severely head-injured patients are exposed to high concentrations of a neurotoxic amino acid for days following injury and thus may benefit from antagonist intervention.

KEY WORDS • traumatic brain injury • glutamate • cerebrospinal fluid • excitotoxicity

Excitatory amino acids (EAA’s) normally function as neurotransmitters but are known to be neurotoxic in high concentrations. They are believed to mediate their toxicity by agonist activity at the N-methyl-D-aspartate (NMDA) receptor-gated ion channel, resulting in cellular edema and accumulation of intracellular calcium leading to cell death.7 There is substantial evidence that EAA’s are integral in the pathophysiology of ischemic neuronal injury.1,2,6 They have also been implicated in hypoglycemic injury,13 seizure disorders,14 and chronic degenerative diseases such as Alzheimer’s disease,13 amyotrophic lateral sclerosis,23 and Huntington’s disease.26

Evidence now supports the notion that EAA’s play an important role in traumatic brain injury. In various models of traumatic brain injury, transient but substantial elevations of extracellular glutamate (one of the EAA’s) have been demonstrated.9,10,17,22 It is postulated that concussive injury results in widespread neuronal depolarization and potassium release,15 causing EAA release and further depolarization. The toxic effects of the EAA’s are thus amplified.

The importance of EAA’s in neurological injury following traumatic brain injury is further supported by results of the use of NMDA antagonists. Antagonists used in either the immediate pre- or postinjury period had protective effects in models of traumatic brain injury as determined by endpoints such as neurological function, metabolic parameters (phosphocreatine:inorganic phosphate ratios), preservation of intracellular magnesium concentrations (normally depleted after injury), and reduction of cellular edema.10,19-21

Elevation of EAA’s in the cerebrospinal fluid (CSF) following traumatic brain injury has been reported in humans,4 however, further information is required. Initial enthusiasm for the clinical use of antagonists has been tempered by the suggestion from animal studies that the therapeutic window following injury is brief. Thus, the duration of any EAA release following traumatic brain injury in humans needs to be determined. Information regarding the incidence and degree of EAA release following traumatic brain injury is also relevant prior to contemplating antagonist intervention. Accordingly, we undertook to measure serially CSF concentrations of EAA’s following severe closed head injury in humans.
Clinical Material and Methods

Patient Population

Approval of the study protocol was granted by the St. Michael's Hospital Research Ethics Board. All patients admitted to our institution with a severe traumatic brain injury (Glasgow Coma Scale score of 8 or less) over a 5-month period were included in the study (Table 1). There were 12 patients, nine men and three women, with ages ranging from 18 to 79 years.

Management Protocol

Our approach to these severely head-injured patients included the prompt treatment of systemic injuries and supportive care aimed at cardiovascular stability and the avoidance of hypoxemia. Therapy for raised intracranial pressure (ICP) by mechanical hyperventilation (target PaCO₂ 27 to 33 mm Hg), neuromuscular blockade, osmotherapy, and the insertion of an external ventricular drain was instituted. Intracranial hematomas were surgically removed.

Cerebrospinal Fluid Sampling

Cerebrospinal fluid was freshly collected every 12 hours and immediately frozen; this CSF would otherwise have been drained and discarded from patients in order to control ICP during the 1st week following traumatic brain injury while an external drain was in place. In order to denature any enzymes that might alter the EAA concentration, 250 µl of 0.3 M perchloric acid was added to each 1.0-ml CSF sample. The samples were kept frozen until assayed. High-performance liquid chromatography with electrochemical detection was employed to assay samples for EAA's. For comparison, CSF was obtained from patients giving consent before undergoing procedures that usually involve some loss of CSF and that do not involve probable ischemia, such as elective CSF shunting procedures for normal pressure hydrocephalus.

Results

Sampling of CSF was possible in all 12 head-injured patients, and was continued until the catheter was either nonfunctional or no longer needed for monitoring, or the patient died. Samples were obtained serially through Day 4 in seven patients and through Day 6 in five. The initial, peak, and final concentrations of glutamate, rather than all 12-hour sample results, for each patient are presented in Table 2, and sampled glutamate concentrations in one patient are presented for illustration in Fig. 1. Peak concentrations of glutamate in the CSF of the head-injured patients were significantly different from those in the three control patients: range 14 to 474 µM versus 4.9 to 17 µM (Mann-Whitney U-test for nonparametric data, p < 0.02); mean ± standard deviation of natural logarithmic transformation 4.19 ± 0.89 µM versus 2.35 ± 0.67 µM (Student's t-test of logarithmically transformed data, p < 0.01). In five of the eight patients still being sampled on Day 3, glutamate concentrations were higher than those in the control range.

Discussion

We set out to determine whether significant levels of EAA's would be measured in the CSF of severely head-injured patients. The important observations included: 1) high levels of glutamate were measured in the CSF of most of the traumatic brain injury patients studied while levels were low in control patients; 2) the concentrations measured in the CSF were at a level that is lethal to cultured neurons; and 3) significant concentrations were measured throughout the sampling period which for some patients extended for days after injury.
Cerebrospinal fluid glutamate and traumatic brain injury

The precise etiology of elevated concentrations of glutamate in CSF is uncertain. Large amounts of glutamate are stored in intracellular compartments, and normal release occurs in small amounts as part of synaptic neurotransmission. Excessive release in large amounts has been observed following ischemia, hypoxia, hypoglycemia, and prolonged seizures. More recently, excessive release of glutamate has been described in models of traumatic brain injury. It is conjectured that the release of glutamate in models of mild concussive injury is not based on ischemia but rather on an independent process. This process likely involves widespread depolarization and subsequent lethal and sublethal excitotoxic effects.

Both neuronal ischemia and concussive injury are thus possible etiological factors of glutamate release implied by the high glutamate concentrations in the CSF observed in this study. However, microdialysis studies of models of both ischemia and traumatic brain injury suggest that the elevation of extracellular concentrations of glutamate is transient and does not extend for hours after the insult. Our observation that elevated glutamate concentrations in CSF extend for days after injury differs with this and therefore suggests the following possibilities: ongoing ischemia, or a second phase of glutamate release following either ischemia or traumatic brain injury that occurs in the time frame we observed and beyond the acute time frame of animal studies. Although the incidence of elevated glutamate concentrations in the CSF in the 12 head-injured patients in our series far exceeded the incidence of ischemia anticipated clinically, the incidence of ischemia suggested by postmortem studies of severely head-injured patients ranges from 50% to 90%. Our results therefore are consistent with and supportive of the contention that ischemic neuronal injury is an ongoing process following traumatic brain injury that occurs in a very high proportion of patients and is underrecognized by present monitoring techniques.

The possibility of a delayed release of glutamate following traumatic brain injury is a feasible explanation of our observations, based on the known delayed neuronal death that follows injury and the slow release of various glutamate-containing vesicles known to be in high volume in central nervous system tissue. Other factors must be considered with respect to the timing and degree of glutamate concentrations in the CSF in this study. First, the possibility of contamination by blood or neural tissue exists. Some samples in our study were tinged with blood; however, most were clear. Normal serum levels of glutamate range from 50 to 100 μmol. Given the similar range measured in this study, the samples would be required to be composed of 20% to 100% blood, an unlikely possibility. While transport of glutamate across the blood-brain barrier is possible, these saturable mechanisms are unlikely to be responsible for the high concentrations of glutamate measured in the CSF. One might anticipate neural tissue contamination in initial samples; however, catheters were serially drained and CSF was presumably continually produced, thus exchanging ventricular CSF twice daily.

The kinetics of glutamate transfer from the interstitial space to CSF are not well known, especially in recently injured and edematous brain. However, the available information regarding small hydrophilic molecules, such as glutamate, suggests that equilibration or movement between the extracellular fluid and CSF compartments in both directions is quite rapid (<30 minutes). The half-life of glutamate in the CSF is in the range of 5 to 6 hours. Thus, given the time frame of this study (sampling CSF every 12 hours), the CSF glutamate concentrations measured are a fairly faithful representation of the concentrations that are present in the brain extracellular fluid. That the equilibration time of glutamate between the brain extracellular fluid and CSF is short and that clearance from the CSF is long implies an almost continuous exposure of neural tissue to the high concentrations of glutamate measured.

The neurotoxicity of glutamate has been established experimentally with different approaches. For example, microinjections of glutamate in rat hippocampus results in neuronal death. Neuronal cell cultures briefly exposed to glutamate suffer cellular edema and cell death. Glutamate antagonists prevent such sequelae. The concentration of glutamate required to induce cell death is approximately 100 μM. The release of glutamate into the extracellular space in certain brain regions following traumatic brain injury would result in diffusion down a concentration gradient and in mixing with CSF and extracellular fluid from other regions. Thus, the concentrations in the CSF measured in this study presumably represent a lower concentration than at the source. The peak glutamate levels in the CSF in our group of patients ranged from 14 to 474 μM; clearly, this represents concentrations at the interstitial space sufficient for neurotoxicity.

### TABLE 2
Concentrations of glutamate in CSF of 12 severely head-injured patients and three control patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Glutamate Concentrations in CSF (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td><strong>head-injured patients</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23 (5)</td>
</tr>
<tr>
<td>2</td>
<td>6.1 (8)</td>
</tr>
<tr>
<td>3</td>
<td>31 (4)</td>
</tr>
<tr>
<td>4</td>
<td>50 (15)</td>
</tr>
<tr>
<td>5</td>
<td>49 (36)</td>
</tr>
<tr>
<td>6</td>
<td>72 (5)</td>
</tr>
<tr>
<td>7</td>
<td>21 (13)</td>
</tr>
<tr>
<td>8</td>
<td>8.8 (12)</td>
</tr>
<tr>
<td>9</td>
<td>96 (17)</td>
</tr>
<tr>
<td>10</td>
<td>36 (6)</td>
</tr>
<tr>
<td>11</td>
<td>121 (10)</td>
</tr>
<tr>
<td>12</td>
<td>30 (5)</td>
</tr>
<tr>
<td><strong>control patients</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.9</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

*CSF = cerebrospinal fluid. The number of hours after injury when each sample was obtained is presented in parentheses.
The observations in this study of concentrations of glutamate in the CSF imply that the severely head-injured patients are suffering glutamate neurotoxicity days after injury. This is a provocative notion. However, other mitigating factors, such as the concomitant release of inhibitory neurotransmitters or altered receptor affinity possibly due to lowered extracellular pH, may be important. Nevertheless, our ability to measure this level of glutamate sampled from a lateral ventricle suggests that toxic concentrations threaten tissue in regions of the brain distant from initial injury.

The clinical significance of these observations is not known. The relationship between the degree of glutamate concentration elevation in CSF and outcome was neither hypothesized nor tested in this study because of the small number of patients. However, the successful use of glutamate antagonist in animal models of head injury has led to the suggestion of antagonist intervention in human head injury. The results of this study indicate that a more extensive study to determine the clinical significance of CSF glutamate concentrations is warranted and would provide the rational basis for assessing a trial of antagonist intervention in humans.

Conclusions

We observed high CSF concentrations of glutamate in severely head-injured patients, which implies that neural tissue is being exposed to concentrations that are neurotoxic in vitro, regardless of the etiology of such levels. These levels of glutamate, combined with the time frame over which we observed them, suggest an important opportunity for intervention in a clinically relevant therapeutic window.

References