The Role of Excitatory Aminoacids and its Neurotoxic Impact in Severe Head Injury Patients


Abstract: Background: Knowledge on metabolic aspects is essential for a better understanding of new pharmacological avenues and non-pharmacological strategies. Traumatic brain injury results in changes the composition of cerebrospinal fluid (CSF), which may represent a unique clinical window on brain pathophysiology. The role of excitotoxicity in mechanically-induced cell death and the molecular events that excessive release of glutamate induce, including apoptosis and delayed inflammatory processes, are shown to be vital to determine the outcome of the head injury. Besides, brain injury induces an inflammatory response and production of oxygen-derived reactive species which affect many organs including heart, brain, kidney and gastrointestinal tract. Method: Lumber CSF obtained from 33 patients with severe head injury was collected and HPLC was used for the quantification of aspartic acid and glutamic acid. Kit methods were used for the determining the enzyme activity of LDH, AKP, SGOT, SGPT and CPK by Microlab instrument. For comparison, CSF samples from 32 healthy subjects without any neurological defects were used. Similar protocol was followed for both patients and healthy subjectsResults: Aspartic and Glutamic acid are both increased significantly in patients with severe head injury. Among the enzyme analyzed, LDH and CPK both are increased and s-GPT or ALT decreased significantly. There is no change observed in AST and AKP.Conclusion: From this study besides the role of excitatory amino-acids, the impact on enzyme activities shows a clear sign on the affect of cardial enzymes due to severe head injury. This information may be of vital importance in treating these patients with antagonists in suppressing the levels of glutamic and aspartic acids.

Key words: Aminoacids · Head Injury · Cerebrospinal fluid

INTRODUCTION

Traumatic head injury is an insult to the brain from an external mechanical force, leading to temporary or permanent impairment of cognitive, physical and psychological functions. In more than half of trauma related death, head injury contributed significantly to the outcome [1]. It is estimated 180 to 220 per 100 persons per year in USA and in the case of severe head injury, mortality can reach as high as 35-70%. Among the gender males are affected more than twice as often as females [2]. Road Traffic Accidents involving motor vehicle drivers, cyclist and pedestrians are the major causative factors for head injuries. Falls from roof, stairs causes head injury in elderly and children, athletes especially foot ball players are subjected to head injuries very often.

In brain injury, both direct impact and countercoup injuries can result in focal bleeding beneath the calvarium and this bleeding can result in contusion,
extracerebral hemorrhages or primarily subdural and epidural [3]. After traumatic brain injuries (TBI) the brain is bathed with potentially toxic neurochemicals and catecholamines surges have been documented in the plasma and cerebrospinal fluid (CSF) of patients with head injuries [4,5]. CSF concentrations of neuron-specific enolase (NSE) and S100B have also shown a promise as markers of brain injury, but it again shows limitation in children and infants [4]. Besides, head injuries causes release of free radicals and breakdown of membrane lipids and these lipids fragment into mediators of inflammation [5]. Generally the excitotoxic aminoacids (glutamate, aspartate) initiate cascade of processes culminating in an increase in intraneuronal calcium and cell death [6,7]. Magnesium is also reported to work at the post synaptic receptor to reduce the neurotoxic effect of glutamate [8]. Although the status of neurotransmitters is not well understood in TBI, disturbances in the function of these substances may underlie certain problems that follow TBI, such as mood, behavior or intellect problems. Medications may be used to try and normalize function and consequently improve symptoms [7].

Generally, Glasgow Coma Scale (GCS) and Glasgow Outcome Scale (GOS) are widely used clinical scoring systems to measure the severity of neurological injury after TBI, but both have limitations in infants and small children. Prostaglandins, inflammatory mediators produced by membrane lipid breakdown, are also elevated dramatically in plasma of patients with moderate to severe head trauma during the first two weeks after head injury. Among the other possible markers the excitatory amino-acids glutamate and aspartate usually function as neurotransmitters in CNS but their role in head injury is not well emphasized [9-12]. With no neuroprotective drug has been beneficial in improving the outcome of severe TBI nor has any prophylactically-induced moderate hypothermia shown any beneficial effect on outcome in severe TBI, despite the optimism generated by preclinical studies [13]. Despite recent advances in our understanding in pathophysiology of TBI, we still need effective neuroprotective agents. In our study, thirty patients in coma from a severe head injury underwent monitoring of CSF concentrations of glutamate and aspartate by HPLC technique [14] during the first 24 hours after injury. The main aim has been to determine the contents and dynamics of excitatory amino acids (EAAzs), glutamate (GLU) and aspartate (ASP) in the CSF of patients with acute head injury and to clarify the relationship of EAAzs with clinical features and outcomes. The study is also directed to study the impact of EAA on enzyme activity in the brain such as LDH, ALT, AST, CPK and ALK.

MATERIALS AND METHODS

This is a comparative/observational study. After approval from Ethical Review Committee, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, study was conducted in the department of Neurosurgery, LUMHS Jamshoro. 33 patients with head injury in unconscious condition with Glasgow Score 8-10 were included in this study. GCS of the patients were recorded and the CSF samples were collected from patients via lumbar puncture (keeping in mind no midline shift or supra or infra tentorial masses on C.T. scan). CSF from 30 healthy controls was also collected during lumbar anaesthesia but without neurological disease. All samples were collected in 2 ml test tubes and stored at 40°C and analyzed for the glutamate and aspartate. SPSS version 16 software was used for data analysis. Critical value of ‘p’ indicating the probability of the significant difference was taken as 0.05 or less for all the comparisons.

Clinical Information: Thirty three TBI patients consisted of 7 women; average age, 35 years, age range, 16-70 years within 24 hours after head injury. Any patient having a history of cerebrovascular diseases and epilepsy was excluded. 28 patients underwent craniotomy, 2 received continuous ventricular drainage and 3 had non-surgical treatment. Severity of injury for patients group was based on the Glasgow Coma Scale (GCS) result and the outcome on the Glasgow Outcome Scale (GOS) showed between 8-10 Grade. In healthy control group, 8 women; average age, age 41 years; age range (19-67 years) without any neurological disorders were included.

RESULTS

Table 1 showed the clinical data of the TBI patients and control group. CSF protein is almost entirely constituted of albumin, but small amounts of globulins may also be found. However, when the nervous system is affected, the protein levels may be increased, as reported in 1997 by Tuduly et al. [15] in dogs with canine distemper.
Table 1: Clinical Data on patients with severe head injury and healthy controls. The value of CSF total proteins and albumin is given in pg/ml ± SEM.

<table>
<thead>
<tr>
<th>Patient</th>
<th>n</th>
<th>Females</th>
<th>Age</th>
<th>Total Proteins</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control</td>
<td>32</td>
<td>8</td>
<td>36±2.5</td>
<td>66.8±1.5</td>
<td>423±1.8</td>
</tr>
<tr>
<td>TBI</td>
<td>33</td>
<td>7</td>
<td>29±1.7</td>
<td>410.3±56.8***</td>
<td>156.8±4.6***</td>
</tr>
</tbody>
</table>

***p <0.001

Table 2: CSF levels of Aspartic and glutamic acid in Controls and TBI patients. All values are given as mean ± SEM in µmol/L.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Controls</th>
<th>Head Injured Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>1.45±0.07</td>
<td>3.78±0.16***</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>19.10±0.54</td>
<td>37.17±1.55***</td>
</tr>
</tbody>
</table>

***p <0.001

Table 3: CSF Enzyme activity of LDH, ALT, AST, CPK and AKP in healthy control and TBI patients, all values are shown as mean ± SEM.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Controls</th>
<th>Head Injured Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>108.8±7.50</td>
<td>419.4±40.73***</td>
</tr>
<tr>
<td>SGPT or ALT</td>
<td>26.43±1.67</td>
<td>18.63±1.12***</td>
</tr>
<tr>
<td>SGOT or AST</td>
<td>23.03±1.48</td>
<td>25.90±1.69</td>
</tr>
<tr>
<td>CPK</td>
<td>27.27±1.47</td>
<td>40.17±1.97***</td>
</tr>
<tr>
<td>ALK</td>
<td>25.03±1.11</td>
<td>28.20±1.62</td>
</tr>
</tbody>
</table>

***p <0.001

The concentrations of Glu and Asp were 19.1±0.54 and 1.45±0.067 µmol/L respectively in CSF and in patients with TBI were 37.17±1.55 and 3.78±0.16 which were significantly higher than those in the controls with p <0.001 (Table 2).

Table 2 showed the mean concentrations of Glu and Asp in CSF in the TBI group which were significantly higher than those of the control group (For both Glu and Asp, p <0.001). In patients admitted within 24 hours after severe injury (n=13), peak Glu values appeared within 24 hours in all patients and the mean value remained higher than control values.

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Table 3 showed the enzyme activity of LDH, ALT, AST, CPK and AKP in both groups. The results showed significant elevation of LDH and CPK both related to heart function and decrease in ALT related to liver function where as both AST and ALK remained unchanged.

**DISCUSSION**

In Western societies like Europe and USA, Traumatic brain injury (TBI) represents an extreme social-economic burden, expected to become the third leading cause of mortality until 2020 [16]. TBI is the leading killer after trauma, in part because of coagulopathy, resulting into persistent mental disturbance and cognitive deficits [17]. Although the previous studies indicated that oxidative stress and functional deficits occurring after TBI are interrelated events, the knowledge of the mechanisms underlying the developments of such cognitive deficits is still unknown. Recent evidence has clearly demonstrated that traumatic brain lesions are highly dynamic and that the different lesions observe after closed head injury are not single events but process set in motion by the mechanical impact. These processes are not finished until and unpredictable time after injury [18]. Severe TBI causes damage to individual nerve cells (neurons) and loss of connection among neurons which can lead to a breakdown of overall communications among the neurons in the brain. This damage causes a series of reactions that eventually lead to swelling of the axon and disconnection from the cell body of the neuron. In addition the part of the neuron that communicates with other neurons degenerates and releases toxic levels of chemical messengers called neurotransmitters into the synapse or space between neurons, damaging neighboring neurons through a secondary neuroexcitationary cascade. Many of these cells can not survive the toxicity of chemical onslaught and initiate programmed cell death or apoptosis. This process usually takes place within the first 24 to 48 hours after the initial injury, but can be prolonged. It is well established that in severe head injuries, posttraumatic secondary insults, such as brain hypoxia, hypotension or anemia and exacerbate neuronal injury lead to poorer outcome. Experimental and clinical evidence suggested that moderate hypothermia (32-34°C), may limit some to these deleterious secondary metabolic responses. It has been demonstrated good intracranial pressure control and better outcome is the result when compared with patients maintained in normothermia and given conventional treatment. Despite its proven clinical role in neuroprotection, hypothermia research has been
inconstantly followed for various reasons [19]. Experimental data indicated that destructive oxidative events reach their peak within the first 24 hour after trauma in head injury (HI) and that brain damage occurring due to this impact can be the case of death or irreversible permanent disabilities in affected patients [20].

In the healthy brain, the glutamate functions as a neurotransmitter, but and excess amount of glutamate (Glu) in the brain causes neurons to quickly overload from too much excitation, releasing excessive Glu initiating degeneration and programmed cell death. Qureshi et al. have previously shown the role of glutamic acid in PD and other neurodegenerative disorders [21]. The research shows that glutamate reacts with calcium and sodium ion channels on the cell membrane, leading to an influx of calcium and sodium ions into the cell. Glutamate acts both as a primary excitatory neurotransmitter and a potential neurotoxin within the mammalian brain. Evidence indicates that hyperactivity of the glutamate system contributes to neuronal death in brain trauma [22]. Scientists have shown that after diffuse axonal injury neurons can spontaneously adapt and recover by sprouting some of the remaining healthy fibers can develop in such a way that the neuron can resume communication with neighboring neurons. This is a very delicate process and can be disrupted by any of a number of factors, such as neuroexcitation, hypoxia (low oxygen levels) and hypotension (low blood flow).

Traumatic brain injury (TBI), like other central nervous system pathologies causes change in the composition of cerebrospinal fluid (CSF). Consequently analysis of the CSF components is important to better understand the pathological process involved in such diseases. Both excitatory amino acids (EAAs), Asp and Glu are increased in cerebrospinal fluid (CSF) in TBI patients (TABLE 2). Accumulating evidence suggests that the aspartate and glutamate systems are involved not just in fast synaptic transmission, but also in plasticity and higher cognitive functions. Excessive increase in extracellular Glu and Asp have been demonstrated after ischemia, hypoxia, prolonged seizures and TBI and are believed to mediate excitotoxicity by acting as agonists at the N-methyl-D-aspartate and or amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainic acid (AMPA-KA) receptor-gated ion channel, leading to cellular edema and accumulation of intracellular Ca²⁺ and Na⁺⁺, with subsequent lethal and sublethal excitotoxic effects [23]. It has been suggested that concussive brain injury triggers widespread neuronal depolarization, causing release of Ca²⁺-dependent Glu from synaptosomes and further depolarization, thus amplifying the toxic effects of EAAs.

Hence, it can be said that TBI increases extracellular levels of the excitatory amino acid glutamate and aspartate and N-methyl-D-aspartate (NMDA)-receptor antagonists protect against experimental TBI. These two findings have led to the prevalent hypothesis that excitatory amino acid efflux is a major contributor to the development of neuronal damage subsequent to traumatic injury [24]. Thus, it confirms the hypothesis that a wide time window exists for the use of antagonists to reduce excitotoxic brain damage induced by aspartic and glutamic acid. Apart from the role of glutamate, it is also shown that the disturbance in oxidant-antioxidant balance might play a part in rendering the tissue more vulnerable to free radical induced injuries. In healthy individuals, antioxidant activity counterbalances free radical production, but in the case of ischemia, the balance between reactive oxygen species and antioxidant activity is shifted toward free radicals, causing oxidative stress [25]. Hence measurement of EAAs in CSF might be an index available for judging the severity of the trauma. Excess quantities of glutamate and aspartate in the extracellular space may lead to uncontrolled shifts of sodium, potassium and calcium, disrupting ionic homeostasis, which may lead to severe cell swelling and cell death. Knowledge of these pathophysiologic mechanisms will enable scientists to develop new therapeutic strategies, which can function as neuroprotective. The potential of such therapies needs to be worked out to avoid neurotoxic effect generated by elevation of glutamate and aspartate as well as Aspartic acid. The removal of excess glutamate from brain fluids after acute insults such as traumatic brain injury is expected to prevent excitotoxicity and the ensuing long lasting neurological deficits.

Among many important enzymes, Alanine aminotransferas (ALT) is a pyridoxal enzyme found mainly in the liver and kidney, but also in small amounts in the heart, muscle, fat, brain. Serum ALT activities have been used broadly as surrogate markers for tissue injury and disease in human and veterinary clinical settings and in safety assessment of chemicals and pharmaceuticals. Because of its relative abundance in liver, increased serum ALT activity is generally considered indicative of liver damage. Patients with head injury often exhibit cardiovascular abnormalities...
and acute pulmonary edema which are regarded as signs of increased sympathetic nervous system activity [26]. The other enzymes such as CPK, LDH, GOT and, AST were also studied in the cerebrospinal fluid within 24 hours prior head injury. A statistically highly significant increase of enzymes LDH, CPK among these enzymes was observed in the CSF. It is concluded that after brain injuries, LDH and CPK is released into the extracellular fluid of the brain and transported to the CSF, however, reverse is the phenomena in case of ALT. The elevation of CPK activity in emergency diseases can be considered to result from muscle hypoxia due to severe stress and general circulatory failure [27]. And the examinations of enzyme activities in the patients with head injury may become a useful aid to make an outlook of their clinical course and prognosis.

Our results seem to provide indirect evidence for transependymal flow of extracellular fluid in brain oedema. The present study confirmed that CSF CK-BB seems to be a sensitive index of acute brain damage, but it reflects best the extent of CNS tissue disruption rather than the severity of neurological deficits [28].

REFERENCES


