Extracellular glycerol in patients with severe traumatic brain injury

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Objective: To study the factors affecting extracellular glycerol (Gly) in patients with severe traumatic brain injury (STBI).

Methods: Perilesional extracellular Gly and cerebral blood flow (CBF) in 53 patients with STBI were consecutively monitored. Simultaneously, the intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were monitored. The hourly minimum of CPP and CBF and the hourly maximum of ICP levels were matched with the hourly Gly. Gly values were divided into several groups according to regional ICP (<15 mm Hg or >15 mm Hg), CPP (<70 mm Hg or >70 mm Hg), CBF (<50 AU or 50-150 AU) and the outcomes (death or persistent vegetative state group, severe or moderate disability group, and good recovery group).

Results: In comparison with the severe or moderate disability group, the Gly concentration of the death or persistent vegetative state group increased significantly, but CBF and CPP decreased significantly. In comparison with the good recovery group, the Gly concentration of the severe or moderate disability group increased significantly, but CBF and CPP decreased significantly. The Gly concentrations in patients with ICP >15 mm Hg, CPP<70 mm Hg and CBF<50 AU were respectively higher than those of patients with ICP <15 mm Hg, CPP>70 mm Hg and 50 AU <CBF<150 AU. In patients with diffuse axial injury, the mean Gly concentration was (201.17 ± 55.00) µmol/L, which was significantly higher than that of the patients with epidural hematoma (n=7, 73.26 ± 8.37 , P<0.05) or subdural hematoma (n=9, 114.67 ± 62.88, P<0.05), but it did not increase significantly when compared with those in patients with contusion (n=24, 167.48 ± 52.63).

Conclusion: Gly can be taken as a marker for degradation of membrane phospholipids and ischemia, which reflects the severity of primary or secondary insult.

Key words: Traumatic brain injury; Glycerol; Microdialysis

Methods

Patients

The data of 53 patients (40 males and 13 females, their ages ranging 15-60 years, mean=42.00 years ± 14.41 years) with severe traumatic brain injury (STBI) admitted to the Neuroscience Intensive Care Unit in Huanhu Hospital of Tianjin in China within 24 hours after injury were studied retrospectively in this article. The mean time from injury to emergency admission was (5.10 ± 3.51) hours and the mean GCS on admission was 5.40 ± 2.17. The patients received standard aggressive management that included rapid removal of intracranial hematomas, ICP monitoring performed with ventriculostomy, careful management of ICP by means of artificial ventilation, neuromuscular paralysis, mannitol, and ventricular drainage. In a few patients with uncontrolled high ICP, barbiturate infusion or induction of moderate hypothermia was made.
Cerebral microdialysis and biochemical analyses

Only the patients with preexisting ventriculostomies that had been placed to measure ICP were considered for microdialysis. Microdialysis catheters were inserted within 4 hours of admission. CMA 70 microdialysis catheters (CMA Microdialysis, Sweden) were inserted into the cerebral cortex of the perilesion and the relatively normal brain tissues. The catheters, which were designed specifically for use in the human brain under clinical conditions, had a membrane length of 10 mm, a diameter of 0.6 mm, and a molecular weight limit of 19 840 u. The catheters passed the cranium through the burr holes in the bone flap and were tunneled through the scalp, to which they were secured with sutures. Immediately after surgery, the catheters were connected to the syringes placed in CMA 106 miniaturized microinfusion pumps, which were placed on the patients’ chest. The perfusion medium was artificial cerebral spinal fluid, delivered at a rate of 0.3 µl/min. This low rate of flow was chosen in order to obtain about 70% recovery of molecules across the dialysis membrane. The samples were collected at 1-hour intervals.

In the CMA 600 microdialysis analyzer, high-precision pipettes were used to collect the samples and reagents. For glucose, pyruvate, lactate and Gly determinations, the formation rate of a colored substance was measured in a filter photometer at 546 nm. All reagents used were obtained from CMA microdialysis.

ICP, CBF and CPP monitoring

A purpose-built three-lumen cranial bolt was designed to grip and immobilize the ventricular catheter, the microdialysis probe, and the laser Doppler flowmetry probe. The newly-developed polycarbonate bolt did not cause artifacts on CT scan and was compatible with magnetic resonance imaging (MRI). It was designed in such a way that the tips of the microdialysis probe and the laser Doppler flowmetry probe were directed at the place ±10 mm away from the ventricular catheter by angling the lateral lumina of the bolt at 16 degrees. In this way, the possible local brain trauma caused by the ventricular catheter was minimal. The bolt was threaded and tapped into the cranium using a 7-mm twist drill hole and placed under local anesthesia at bedside or in the operating room.

Calculation and statistical analyses

In all the patients, the physiological data such as ICP, CPP, CBF, and clinical characteristics, were input into a computer and these data were time-locked with the biochemical data for analysis. The Gly values were divided into several groups according to the outcomes (A: the good recovery group, B: the severe or moderate disability group, C: the persistent vegetative state or death group), ICP (ICP>15 mm Hg or ICP<15 mm Hg), CPP (CPP>70 mm Hg or CPP<70 mm Hg), and CBF (<50 AU or 50-150 AU). The hourly minimum of CCP, CBF and hourly maximum of ICP levels were matched with hourly Gly concentration. All the data were presented as mean ± standard deviation. All statistical calculations were performed in a computer with SPSS11.5 software. Two-sample t-test for independent samples and one-way ANOVA were used.

RESULTS

Relationship between outcome and Gly, CBF, and CCP

In comparison with the severe or moderate disability group, the Gly concentration of the persistent vegetative state or death group increased significantly (P<0.05), but CBF and CCP decreased significantly (P<0.05). In comparison with the good recovery group, the Gly concentration of the severe or moderate disability group increased significantly (P<0.05), but CBF and CCP decreased significantly (P<0.05, Table 1).

Effect of ICP, CBF and CPP on Gly concentration

The Gly concentrations in ICP>15 mm Hg group, CPP<70 mm Hg group and CBF<50 AU group were respectively higher than those of ICP <15 mm Hg group, CCP>70 mm Hg group and 50 AU <CBF <150 AU group (Table 2).

Comparison of Gly values in CT findings

In the patients with diffuse axial injury, the mean Gly concentration was 201.17 µmol/L ± 55.00 µmol/L, which was significantly higher than that of the patients with epidural hematoma (n=7, 73.26 µmol/L ± 8.37µmol/L, P<0.05) or subdural hematoma (n=9, 114.67 µmol/L ± 62.88 µmol/L, P<0.05), but the Gly concentration of the patients with diffuse axial injury did not increase significantly when compared with that of the patients with contusion (n=24, 167.48 µmol/L ± 52.63 µmol/L).
DISCUSSION

In this study, there were two main findings: 1) The outcome was worse in the patients with high levels of Gly in the dialysate. And 2) the presence of a focal contusion and primary or secondary ischemic events such as low CPP, raised ICP, or reduced CBF, were the clinical features most strongly correlated with the high concentration of Gly.

Methodological aspects

Before detecting cerebral Gly, we had monitored ICP and/or CBF in the patients with STBI for 6 years, some complications were observed, and the worst and common complication was cranial bacterial infections. We estimated the risk of microdialysis to be about 0.5%-2% when performing any intracranial monitoring for 7 days. According to the concept of multimodality monitoring, a combination of several diagnostic methods for neurochemical and neurophysiological parameters in Neurointensive Care Unit was used in this study. By combining microdialysis with continuous ICP, CBF, and CPP monitoring, a more complete picture of the brain injury process was obtained, but the risk of cranial bacterial infection was therefore increased. In order to prevent cranial bacterial infections, the cerebrospinal fluid was collected and examined every day in all the patients. If the cell count was over $1 \times 10^7$ /L with polymorphonuclear cells predominating, glucose less than 30 mg/dL, and protein more than 200 mg/dL, acute bacterial infections likely occurred, the monitorings were stopped. All the patients in this study took antibiotics as clinically determined. We restricted the multimodality monitoring for 3-4 days to lessen the risk of infection.

These samples were omitted from further data analysis: 1) Whenever the patients were accidentally disconnected from the microdialysis pump, ICP probe, or laser Doppler flowmetry; 2) Separately increased Gly levels within 2 hours, and the data next to them were steady; 3) Increased ICP was caused by cough and suction; And 4) the first postreconnection samples contained the dialysis fraction that had been standing in the microdialysis probe during the disconnection period. Several methodological limitations inherent to microdialysis may affect our results. The microdialysis research was limited by providing only focal neurochemical information. The evaluation on patients during 3-4 days after trauma with multimodality monitoring was possible only in selected cases, and despite the consecutive accrual design, the selection bias of severely injured individuals with ventriculostomies in place may limit the general applicability of these results to less severely injured patients.

Gly and cellular membrane degradation

Following the original observation of degradation of membrane phospholipids and liberation of free fatty acid in experimental cerebral ischemia, this neurochemical event has attracted considerable research interest and is believed to account for important features of acute brain injury such as cellular membrane damage and edema formation. Gly is one of the end products of

| Table 1. Relationship between outcomes and Gly, CBF, and CCP |
|-----------------|----------------|-----------------|----------------|
| Groups | n | CCP | CBF | Gly |
| A | 26 | 80.03±6.71 | 123.61±19.60 | 76.59±13.03 |
| B | 11 | 71.63±5.54 | 87.81±19.00 | 132.07±24.53 |
| C | 16 | 57.69±8.70 | 42.56±14.77 | 206.68±25.58 |

*P<0.05, compared with Group B, $t=48.15$; △P<0.05, compared with Group C, $t=51.93$; △P<0.05, compared with Group C, $t=67.12$.
A: The good recovery group. B: The severe or moderate disability group; C: The persistent vegetative state or death group.

| Table 2. Relationship between Gly and ICP, CBF, and CCP |
|-----------------|----------------|----------------|
| Items | CPP (mm Hg) | ICP (mm Hg) | CBF (AU) |
| Samples | <70 | >70 | >15 | <15 | <50 | 50-150 |
| Gly | 162.87±55.98 | 70.02±16.61 | 273.58±26.71 | 133.78±63.79 | 69.94±16.61 |
| t | 78.41 | 52.98 | 44.79 |
| P | <0.01 | <0.01 | <0.01 |
degradation of membrane phospholipids. Because of the relative ease of measuring the water soluble glycerol molecule, Gly, analyzed in brain homogenates, is offered as a useful marker for phospholipid degradation in cerebral ischemia.

The brain is virtually devoid of triglycerides and the increase in microdialysis-Gly observed after acute brain injury is therefore thought to originate from degradation of membrane phospholipids. However, the alternative possibility of formation of Gly from glucose needs to be considered. Even though this pathway requires ATP shortage, more data are required in order to confidently exclude this possibility. Another potential pitfall in microdialysis measurements is leakage of Gly through an injured blood-brain barrier (BBB). This is particularly concerned in Neurointensive Care Unit where plasma Gly may reach high levels owing to a stress-induced degradation of triglycerides and by the administration of exogenous Gly containing infusion solutions. The latter is illustrated in a case report of a TBI patient receiving intravenous Gly as osmotherapy for cerebral edema. Upon intravenous injection of Gly infusion, a dramatic increase of intra-cerebral Gly was observed. To help distinguish a compromised BBB effect from a true intracerebral event, a catheter in the abdominal subcutaneous adipose tissues is used to simultaneously monitor the systemic Gly levels.

**Gly and CT findings**

This research showed that the Gly concentration was higher in patients with contused cortex or diffuse axonal injury. Bullock et al found that extracellular amino acids increase approximately 10-20 times in contused cortex in comparison with noncontused cortex and speculated that neuronal disruption by shearing forces at impact when the contusion occurred leads to development of membrane micropores and leakage of amino acid. Glutamate may then contribute to pericontusional edema and ischemia by inducing the opening of neuronal ion channels to promote potassium efflux and sodium influx. Hillered et al found that the increase of microdialysis-Gly lagged behind the increments of microdialysis-glutamate in several ischemic regions. We therefore speculated that excitotoxicity leads to degradation of membrane phospholipids and higher Gly levels in patients with contused cortex or diffuse axonal injury.

**Gly and secondary ischemia**

Heiss et al and Sarrafzadeh et al found that Gly was correlated to focal CBF in patients with subarachnoid hemorrhage. But the finding of Johnston et al, which was limited by comparing the global BBF with focal biochemistry, showed that Gly in patients with TBI did not change significantly when CPP increased or decreased. Therefore, the relationship between cerebral ischemia and changes in interstitial Gly in patients with STBI needs further illumination. CPP reflects the global BBF and microdialysis monitors the focal biochemistry, but there is great difference in brain regional CBF and neurochemical event in different positions. In order to avoid possibility of inaccuracy when comparing the global BBF with focal biochemistry, we monitored focal CBF with laser Doppler flowmetry probe, too. This research found that Gly was a useful marker for ischemia.

Recently-published data suggest that neurons may take up Gly from the extracellular space and use it as a substrate for cerebral energy production. The concept of Gly being a marker for secondary membrane damage may thus turn out to be a two-edged sword, in which the accumulated Gly may serve as an alternative oxidizable substrate contributing to energy production following brain injury.

**REFERENCES**


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