Ultrastructural and Biochemical Effects of Trauma on Normal and Dehydrated Brain

A Research Study and Review

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Background: In hypertonic dehydration, fluid loss is known to be from the intracellular compartment. Also fluid loss in vascular bed increases the osmotic pressure and results with decrease of fluid flow to these compartments. We applied hypertonic dehydration by fluid restriction to evaluate the effects of dehydration on posttraumatic brain edema and swelling.

Methods: Four groups, each including 10 pigs composed the study group. Group 1: we performed craniectomy and followed up for 1 hour for intracranial pressure (ICP) measurement. Group 2: we performed craniectomy and hypertonic dehydration by fluid restriction for 3 days and followed up for 1 hour for ICP measurement. Group 3: we performed brain trauma with Madsen trauma model. Group 4: we performed brain trauma after dehydration.

Findings: ICP was found to be 10 mm Hg at the first, 6.5 mm Hg at the second group but increased gradually after trauma in group 3. At the fourth group ICP was high at the first 30 minutes. After the experiment brain fluid level was 80.9% at the first, 77.9% at the second, 83.5% at the third, and 82.8% at the fourth groups. Ultrastructural examination revealed normal brain tissue at the first, light degeneration at the second, advanced degeneration at the second. The third group was also highly degenerated but was found to be better than the third group. Brain superoxide dismutase and malondialdehyde levels were found to be increased.

Interpretation: Even though hypertonic dehydration causes minimal cellular degeneration it increases the resistance of brain to trauma and high ICP especially at the first 30 minutes.

Key Words: dehydration, trauma, superoxide dismutase, malondialdehyde, brain edema

MATERIALS AND METHODS

Experimental protocol was approved by the Committee for Animal Research in Çukurova University.
Medical Faculty. The study group consisted of 40 domestic pigs of both sexes, weighing between 16 and 20 kg, with an age of 1 year. Forty of the subjects stayed in separate rooms with sufficient air circulation at a temperature of 27°C for 3 days and were fed with salt-free bread.

Weight, hematocrit (Htc), serum osmolarity, urine density, blood Na levels of all the animals were analyzed at the first and third day before the examination. All the animals were paralyzed with succinile choline 1 mg/kg for intubation and anesthetized with IM 2 mg/kg xylazine and IV 3 mg/kg ketamine and monitored with a Hewlett Packard device for temperature, pulse rate, and respiration. The pigs were divided into 4 groups.

**Group 1**
All the subjects underwent biparietofrontal craniectomy and we introduced 18 G canula to the ventricule through the left parietal to monitor the pressure for 1 hour with Harward Universal Oscillography. At the end of the first hour after sacrificing the subjects with high-dose pentobarbital we removed the whole brain and examined them macroscopically. We removed 2 × 2 × 1 cm brain tissue and transacted it into 3 equal pieces. The first piece was immediately placed in 5% gluteraldehyde buffered at pH 7.4 with Millonig phosphate buffer for 3 hours and postfixed in 1% OsO4 for 2 hours. The tissue pieces were then dehydrated in graded ethanol, embedded in araldite, and processed for electron microscopy using conventional methods. The second piece was immersed in isotonic sodium solution (10% NaCl) for the analysis of Cu, Zn superoxide dismutase enzyme using biochemical techniques. The third piece was prepared for molanalcdehyde analysis.

**Group 2**
In addition to the procedures applied at the first group we restricted fluid uptake for 3 days.

**Group 3**
We performed trauma with Madsen trauma model following the procedures applied to the first group.

**Group 4**
This group underwent trauma and fluid restriction. We analyzed the wet and dry weight of the brain tissue after the removal of brain tissue for biopsy.

**RESULTS**

In water restricted groups fatigue, guide disturbance, and loss of appetite developed at the third day of water inhibition. We observed weight loss of 6%. We detected increase in K+ and Na+ levels (mean 6.5 mm Hg), Htc (8%), urine density (3%), and serum osmolarity (8.6%).

Brain tissue pressure was found to be a mean 10 mm Hg before the experiment whereas it reduced to 6.5 mm Hg at the dehydrated group. We did not observe significant difference in brain tissue pressure at groups 1 and 2. After the application of trauma brain tissue pressure of the subjects in group 3 elevated to 17 mm Hg at the fifth, 23 at the fifteenth, 35 at the thirtieth minutes, and to 42 mm Hg at the first hour, and the rhythm of breathing deteriorated at this measurement point. These values were as follows in the fourth group: 9 mm Hg at the fifth, 13 mm Hg at the fifteenth, 33 at the thirtieth minutes, and 42.2 mm Hg at the first hour.

The macroscopic examinations of the brains (groups 1, 2) were found to be normal. We observed multiple intracerebral hematoma especially at the site of trauma in subjects at the third group. At the fourth group we detected intracerebral hematoma only in 2 of the subjects. We observed bilateral intraventricular and subdural hematoma in all the subjects at groups 3 and 4.

Dry and wet weights of the brains were evaluated to assume the water content of the brains. It was found to be 80.9% at the first, 77.9% at the second, 83.5% at the third, and 82.8% at the fourth group.

**ULTRASTRUCTURAL FINDINGS**

**Group 1**
Ultrastructural examination of this group revealed normal nerve cells, glial cells, and nerve fibers.

**Group 2**
Although slight-to-moderate shrinkage of the nerve cells and widening of pericapillary interstitial spaces were observed, the ultrastructural observations were similar to those group 1.

**Group 3**
Degenerative changes were commonly seen in this group. The nerve cells displayed nuclear chromat in clumping and swelling of the mitochondria with dissolution of the inner membranes and enlargement of the endoplasmatic reticulum and golgi cisternae (Fig. 1). Similar structural alterations were also noted in glial cells. Although, some of the myelinated nerve fibers showed normal structure, most of them exhibited disruption and separation of the myelin sheath lamella. The capillary endothelial cells disclosed nuclear and cytoplasmic abnormalities. Furthermore, excessive edematous areas were noted at the pericapillary zones.

**Group 4**
The brain ultrastructure were similar to group 3, but degenerative changes were less extensive. On the other hand, the cell structure was comparable with group 2. Although some of the nerve cells revealed nuclear and cytoplasmic alterations (Fig. 2), most of the nerve and the glial cells showed minimal structural abnormalities. Although some of the myelinated nerve fibers exhibited slight-to-moderate myelin sheath alterations (Fig. 3) similar to those of group 3, most of the fibers showed normal ultrastructure in general. Furthermore, the capillary endothelial cells displayed normal structure, but the pericapillary edematous areas were also noted.
SOD AND MDA LEVELS

Average SOD levels of the groups were as follows; in group 1: 2258.2, in group 2: 2633, in group 3: 5654.2, and in group 4: 3481.6 μ/g.

Malondialdehyde (MDA) levels were found to be 0 nmol/mL in group 1, 10.54 nmol/mL in group 2, 24.6 nmol/mL in group 3, and 12.6 nmol/mL in group 4.

DISCUSSION

Secondary cerebral edema in addition to the primary intracranial pathology threatens life besides creating difficulty in reaching the primary pathology during surgery. The main aim of treatment in brain edema and swelling is to move the fluid from intracellular and interstitial compartments to the vascular bed and than discharge with urine. We also need to repair the cellular permeability to provide stable osmolarity, which depends on the pressure gradients of the various compartments. Cerebral spinal fluid drainage, hyperventilation mannitol, dexamethasone are all used with these aims. However, to move the fluid from intracellular and interstitial compartments to the vascular bed with these procedures and agents takes a long period of time. Hypertonic dehydration can be produced by preoperative restriction of fluid intake for 3 days.5,6,8,10,12,23,32 In this type of dehydration two-third of the fluid drawn to the vascular bed is from the intracellular compartment, which includes 10 times the total brain fluid. This results in cellular shrinkage, relaxation in brain tissue. Both facilitates retraction of the brain and thus management of the primary pathology and reduces the traumatic effect of retraction on any cellular area.2–4,6,8,9,11,13–15,23,33–35 Yaşargil36 restricts fluid intake for 3 days before vascular surgery. Paczynski et al8 estimates that brain edema and hydration of the body are correlated and proposes inhibition fluid, and electrolyte intake in patients with trauma, and ischemic brain lesions.

Up to our knowledge, there is not an experimental dehydration model reported in the literature. We inhibited fluid intake of subjects for 3 days to maintain dehydration. Fluid inhibition for 3 days resulted with increase in body temperature, fatigue, anorexia, and guide disturbance, increase in blood Na+ level, Htc, urine density, serum osmolarity, and body weight (6%) decreased in dehydrated subjects. We can suggest that changes in antidiuretic hormone and aldosterone secretion are important factors in the development of this state.8,20,23,37

In our study, the wet and dry weight of normal brain decreased from 80.9 to 77.9 in dehydrated brain and to 83.5 in undehydrated traumatized brain at the first hour after trauma, and to 82.8 in dehydrated traumatic brain.

The mean pressure of the normal brain tissue was found to be 10 mm Hg. After trauma it increased progressively to 17 mm Hg at the fifth, 23 at the fifteenth, 35 mm Hg at the thirtieth, and to 42 at the sixtieth.
minute. Respiratory disturbances and arrhythmias developed at this level. In dehydrated and traumatized subjects initial intracranial pressure (ICP) value was found to be 6.5 mm Hg and increased to 9 mm Hg at the fifteenth minute, 13 mm Hg at the thirtieth minute, 33 mm Hg at the thirtieth minute it was found to be at the same level with hydrated traumatized brain.

These findings reveal that dehydrated brain is more resistant to trauma. This can be explained with diminished effect of trauma on shrunken cells and elevation in intracellular fluid occurs. We did not observe an important difference in pressures between the 2 groups at 30 minutes. We created subdural and intraventricular hematoma in all the subjects in groups 3 and 4. We detected multiple intracerebral hematoma in group 3, but in group 4 we observed minimal intracerebral hematoma in some areas of only 2 subjects. Rare production of intracerebral hematoma in one group can be explained with resistance of dehydrated brain to trauma and dehydration together with hypercoaguability. Labato and coworkers reported that the earliest appearance of hemorrhagic lesions occurs at the first hour and edema is maximum level at the eighteenth hour. However, in our study, hematoma and swelling developed just after the trauma and this is probably due to the intensity of the applied trauma.

After trauma, free oxygen radicals produced by the damaged brain tissue destroys the surrounding intact neural and neurovascular tissue. Free oxygen radicals are removed spontaneously or transformed to hydrogen peroxide with SOD to inactivate the harmful effects. We analyzed the tissue SOD levels. The mean SOD level of the normal brain tissue was found to be 2258.2 μg/g whereas it was 2633.6 in dehydrated brains. In dehydration reduction in regional blood flow is not to significant levels so this finding may indicate the cellular stress due to dehydration and increased antioxidant production resulting with increased production of SOD which has a scavenger effect. Electron microscopic examination of dehydrated brains revealed more degeneration in comparison to the control group. Mean SOD level of the traumatized brain (group 3) was 5654.2 μg/g whereas it was found to be highly decreased (3481.6) in traumatized and dehydrated brains. This finding emphasizes the correlation between the SOD level and tissue damage. This was found to be in concordance with the electron microscopic findings: endoplasmic reticulum cisterna of neuron nuclei were found to be intact and degenerative changes were found to be mild in group 4 in comparison with the third group which showed highly degenerative changes.

Besides SOD levels we also analyzed the level of MDA, superoxidation product of hydrogen peroxide. MDA levels were found to be parallel with tissue damage and SOD levels. It was found to be in immeasurable limits in normal brain but it increased to 10.5 in dehydrated brains and to 12.6 in dehydrated and traumatized brains in concordance with SOD levels. It was 24.6 in normal traumatic brains. Reduction in water content of the brain tissue results in resistance to trauma and reduction in degeneration after trauma.

As a result we can postulate that the following clinical correlations:
(1) Dehydrated tissue is more resistant to trauma in comparison to normal tissue.
(2) SOD and MDA levels are in direct relationship to tissue damage.
(3) Dehydration results in cellular stress with a resultant increase in antioxidants and degeneration.

REFERENCES


