Research article

Characteristics of structural and functional alterations following traumatic brain injury in neurons and glial cells of the sensorimotor cortex

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ABSTRACT

Introduction and Aim: Traumatic brain injury (TBI) is regarded as a significant worldwide health issue and a significant contributor to mortality and disability. The objective of this study was to examine the parameters and nature of the regenerative and destructive processes that occur in the rat cerebral cortex depending on the degree and time of the TBI.

Materials and Methods: The experiments were carried out on 24 sexless adult mice weighing 180–220 g. The primary group of mice had severe TBI. To assess the severity of the TBI model, histological data, animal survival rates, and motor and cognitive dysfunctions were examined. Both light and electron microscopy were used to study the animal brains in each group.

Results: The areas of injury were filled with blood, and microscopic examination revealed that the foci of contusion had destroyed brain tissue in the form of tissue and blood vessel fragments. Most dystrophic neuronal changes in mice with severe TBI between 1 and 21 days after the injury were acute neuronal swelling, hydropic dystrophy of nerve cells with clear cytoplasmic vacuolization, localized and complete chromatolysis, and homogeneous cytoplasm.

Conclusion: Brain tissue lesions develop in the early stages of a TBI through rapid necrotic cell death.

Keywords: Brain tissue lesions; development; traumatic brain injury; apoptosis; necrosis; neurons; glial cells.

INTRODUCTION

Traumatic brain injury (TBI) is regarded as a significant worldwide health issue and a significant contributor to mortality and disability (1). TBI is one of the most frequent forms of injury, making up around 40% of all injuries. TBI is expected to occur in 69 million people annually (2, 3). TBI has been linked to higher rates in children (0–12 years old), adolescents, and young adults (15–24 years old). Even among the elderly (>65 years), there is a significant frequency (2, 4). Despite the growing number of TBI cases, the death rate is lower because patients with TBI have a higher rate of disability (5).

TBI is known to cause neuronal death and gradual atrophy of brain tissue (6). Due to the activation of several pathogenetic pathways, including excitotoxicity, inflammation, and apoptosis, TBI is incredibly challenging for study (7). It has not yet been completely demonstrated how the spatial and temporal characteristics of the ratio of apoptotic and necrotic processes in the pathogenesis of TBI provide an indication of the severity of the inflammatory process at an early stage and apoptosis at later stages. Because of this, the objective of this study was to examine the parameters and nature of the regenerative and destructive processes that occur in the rat cerebral cortex depending on the degree and time of the TBI.

MATERIALS AND METHODS

The experiments were carried out on 24 sexless adult mice weighing 180-220 g. The primary group of mice had severe TBI. There were 30 healthy control mice that were not subjected to experimental TBI.

Device for Simulation of Craniocerebral Trauma of Different Severity, a mechanical spring-percussion device, was used to administer the shock, allowing the shock's force to be adjusted. To assess the severity of the TBI model, histological data, animal survival rates, and motor and cognitive dysfunctions were examined. Both light and electron microscopy were used to study the animal brains in each group.

TBI was applied to the soft covers of the fixed rodent head. The rat was laid in the abdominal position. The animal was immobilized by pressing the limbs with a special fixation device against a wooden platform. The impact device was applied once to the right parts of the head. Different shapes and weights of tupos were used in simulating the injury: rounded, triangular square, and oblique, which reproduced the diffuse and focal mechanisms of the trauma that occurs in human.
trauma. The square shape and truncated shape simulate focal closed and open TBI, while the truncated shape has depressed skull fractures.

The described device was used in experiments on mice and allowed us to obtain standard cranial trauma, as evidenced by the following: when stretching the spring from 5 and above the mark on the scale, severe closed and open cranial trauma, which manifested as loss of consciousness for 20 minutes or more, tonic-clonic seizures in limbs, and rapid and shallow breathing, was caused. After coma recovery, the animals remained disoriented and lethargic for several days; their eating and social behavior were impaired, and all of them had persistent left-sided hemiparesis. Some animals died within the first 15–20 minutes.

Sectional examination revealed bruises in the soft tissues of the parietal skull on both sides; macroscopically massive subarachnoid hemorrhages, multiple depressed fractures, intracerebral hemorrhages, and cerebral edema were noted. For histological examination, the brain was carefully extracted from the cranial cavity, and the condition of the meninges, topography, hemorrhages, and localization of apparent foci of contusion were evaluated macroscopically (Fig. 1).

Mice in the experimental and control groups had their brains removed 1, 7, 14, and 21 days following the injury. Mice were euthanized through an intraperitoneal injection of sodium thiopental (100 mg/kg). The brain was taken from the skull as soon as it was decapitated, rinsed in physiological solution, and then put in a 5–10% neutral buffered formalin solution with a pH of 7.2–7.4 after being immediately opened. After being treated to alcohol at increasing concentrations (70°, 80°, 90°, 96°, and 100°), 0.5–1 cm² frontal brain slices were eventually embedded in paraffin using the technique suggested for light microscopy. The prepared blocks were then divided into slices, each between 5–7 microns thick, and stained with hematoxylin and eosin. The existence of qualitative morphological alterations in the brain tissues was checked using a light microscope (Carl Zeiss, Germany).

Within five minutes of letting the mice out of the experiment, samples of brain tissue from the damaged and unaffected hemispheres were taken for transmission electron microscopy analysis. The tissue fragments were first fixed in a solution of 2.5% glutaraldehyde, then in a solution of 1% osmic acid in phosphate buffer at pH 7.2. The substance was dehydrated in alcohol with increasing levels of alcohol before sealing within an epoxy resin combination (epon-araldite). Ultrathin slices were contrasted using uranyl acetate and lead citrate solutions to improve contrast before being studied under an electron microscope, the PEM-100 (Hinds Instruments, United States). We created semi-thin slices of epoxy blocks up to 1 m thick, stained with hematoxylin-eosin and toluidine blue, and examined them via a light-optical microscope called Optron (Carl Zeiss, Germany) and a thorough evaluation of the processes investigated in the brain tissue. The processes found in the brain tissue were identified using morphometric processing of semi-thin slices. In a light-optical morphological analysis of the frontal sections of the rat cerebral cortex, reactive, dystrophic, necrotic, compensatory-restorative, and apoptotic signs were evaluated.

After counting, the digital data was examined using statistical methods. The I.K. Akhunbaev Kyrgyz State Medical Academy Bioethics Committee approved the study and upheld the confidentiality of the data collected (Protocol No. 1, dated May 30, 2007).

**RESULTS**

The areas of injury were filled with blood, and microscopic examination revealed that the foci of contusion had entirely destroyed brain tissue in the form of tissue and blood vessel pieces. In the frontal regions of the mice cerebral cortex, we examined for reactive, dystrophic, necrotic, compensatory-restorative, and apoptotic signs using photo-optical and electron microscopy. Both the damaged and unaffected hemispheres’ cortical and subcortical

![A: Macroscopic picture of the brains of experimental animals with B: traces of trauma](image-url)
regions were sampled for microscopic analysis. No brain injury was seen in the donor animals of the control group (30 animals), as predicted (Fig. 2).

The areas of injury were filled with blood, and microscopic examination revealed that the foci of contusion had entirely destroyed brain tissue in the form of tissue and blood vessel fragments. Acute neuronal swelling, hydropic dystrophy of nerve cells with evident cytoplasmic vacuolization, localized and complete chromatolysis, hyperchromatism, and cytoplasm homogeneity were the most frequent dystrophic neuronal alterations in mice with severe TBI between 1 and 21 days after the injury.

Around the sites of TBI and in other regions of the brain, changes such vacuolization and enlargement of the perivascular and perineuronal spaces occurred (Fig.3). Diffuse cerebral edema was the cause of these modifications. Additionally, astrocytes and oligodendrocytes’ cytoplasms showed indicators of hydropic swelling (Figs.4 and 5). Additionally, there were signs of increased microcirculatory arteries and their blood filling throughout the brain tissue (Fig.6).

On day 7, dystrophic changes were seen in most neurons as homogenization and wrinkling of the cytoplasm, reduction of processes, hypochromia of the nuclei, and marginal chromatin condensation (Fig. 6). These changes also showed signs of apoptosis. Detectable cellular reactions in the form of apoptosis of neurons and glial cell elements were observed from day 7 and were maximally visible by day 21 (Figs. 6 and 7).
So, mice with severe TBI had rough dystrophic changes in their neurons and glia from day 1 to day 21, and their apoptosis started on day 7. Distant areas of the brain's substance as well as the sites of traumatic damage showed alterations that were indicative of prevalent cerebral edema. Additionally, the brain tissue had indications of microcirculatory capillaries dilating and filling with blood. The phenomenon of neural regeneration was not seen during the observational period.

DISCUSSION

A qualitative investigation and comparative evaluation of the pathomorphosis of experimental TBI under natural circumstances was performed to quantify the pathological changes in the cerebral cortex. The majority of severe TBIs were found to have diffuse lesions throughout the entire study period (1, 7, 14, and 21 days), which were characterized by persistent dystrophic-destructive changes in a significant number of neurons without any evident reparative regeneration during the post-traumatic recovery period. Apoptosis-type cells achieved their greatest significance in the group with severe TBI after 21 days.

The nature and extent of the injury directly affects the temporal and geographical aspects of apoptosis in the brain following a TBI. Apoptotic cells were seen close to necrotic cells, the site of damage, in both experimental and clinical TBI. We have demonstrated that following TBI, neurons, astrocytes, and oligodendrocytes all undergo necrosis and death. Indicators of apoptosis in neurons and glia cells included nuclear and cytoplasmic condensation and the presence of apoptotic cells.

Our findings are consistent with those of other studies that looked at the temporal aspects of the onset of specific destructive (necrosis and apoptosis), reactive, and reparative processes in the foci of brain tissue damage and at a distance, as well as the timing of their onset, peak development, and reversal in experimental animals and TBI victims (8-10). Mice, and humans have all demonstrated that a TBI causes cell death by apoptosis (11). The study (12) is an exception, in which the authors examined whether the degree of brain damage is a factor that initiates neuronal death. Their findings demonstrate that following moderate to severe stress for 24 hours, apoptosis occurs in animal brain tissue.

CONCLUSION

According to the findings of our study, brain tissue lesions develop in the early stages of a TBI through rapid procedures of necrotic cell death. Apoptosis and necrosis processes occur together, and together with immunological inflammation, they greatly contribute to overall neuronal losses and subsequent neurodegeneration.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES