EXPERIMENTAL MODEL OF TRAUMATIC SPINAL CORD INJURY AND NEUROPROTECTIVE EFFECT OF KETAMINE IN ACUTE PHASE OF INJURY

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Objective. To present experimental model of traumatic spinal cord injury and to assess the efficacy of ketamine for neuroprotection in multimodal treatment for spinal cord injury in acute phase.

Material and Methods. The study was performed in 60 rabbits with modeled acute spinal cord injury. The standard open spinal cord injury was inflicted in the lower thoracic spine with graduated impact strength and area using impact device. Further, the multimodal therapy was conducted. Motor function, reflexes, pelvic organ function, and skin sensitivity were assessed. Experimental animals were divided into several groups depending on ketamine therapy start time.

Results. The presented model of spinal cord injury is reproducible, graduated, same-type and similar to clinical injury. The model enables mastering the treatment for spinal cord injury sequelae. The method is easy to study and use, and does not require complex equipment. The study showed significantly better recovery of the motor function after early beginning of ketamine therapy.

Conclusion. Ketamine is an effective neuroprotectant in spinal injury, and its administration in the acute phase of traumatic spinal cord injury improves the results of treatment and prognosis.

Key Words: spinal cord injury, experimental model, ketamine.

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Traumatic spinal cord injury is one of the urgent problems of neuroscience and neurosurgery, since this type of injury leads to both patient suffering and serious social and economic consequences. Spinal injury is not the largest group, but it is particularly important due to the exceptional importance of structural damage, the complexity and severity of its consequences, difficulties in treatment and rehabilitation, high level and degree of disability of injured persons [1].

The development of new methods for treatment and rehabilitation of spinal cord injury is impossible without reproducible experimental model. Ideal model should meet the following requirements:

1) to simulate injury similar to that in patients with spinal cord injury;

2) to be manageable, reproducible, and stable;

3) rely on the technique, which is easy to master;

4) the equipment used to create the model should be simple and capable of standardization [6].

Currently, there are several experimental models of spinal cord injury, which can be classified into the following groups:

1) models using ischemia – reperfusion (by compression or occlusion of the aorta) [8, 13, 15];

2) injury models obtained by applying traumatic impact:

– injury by falling objects having predetermined weight [6];

– improved models with standardization of inflicted traumatic impact [16];

– models using pneumatic [19] and electromagnetic devices;

– traction model [2, 17];

– other options of mechanical action on the spinal cord (finger, scalpel handle, Kocher clamp, surgical forceps, inflatable balloon placed to the epidural space) [11, 12];

3) models with spinal cord transsection [9];

4) photochemically induced spinal cord injury [18].

Reviews show that each model has its positive and negative features, and therefore adequate, reproducible, and technically appropriate model is required.

Morphological examination of the injured spinal cord suggests that tissue damage is not limited to the area subjected to the destructive force, but it continues in time and involves initially intact parts of the brain, resulting in formation of lesion, which is larger than the initial injury. The modern concept of the pathogenesis of traumatic spinal cord

injury considers two main interrelated cell death mechanism: apoptosis and necrosis. Morphological study of spinal cord injury and finding the ways for its recovery showed that both types of cell death occur in the injured spinal cord. Currently, apoptosis is believed to be the most common type of cell death and one of the most important pathways of cellular metabolism after injury. Apoptosis is triggered immediately at the moment of injury, continues for a long time after the initial injury, and extends to a considerable distance from the necrotic contusion focus along the spinal cord. This process manifests as extensive degeneration of nerve fibers along the nervous system. Apoptosis of neurons leads to progressive loss of the number of active cells and glial apoptosis prevents survival and outgrowth of the remaining fibers, resulting in the absence of adequate regeneration in the spinal cord [5].

Spinal cord injury results in increased level of the excitotoxic amino acids outside the cell [7]. There are five subtypes of glutamate-activated receptors, NMDA subtype (N-methyl D-aspartate receptors) being the main subtype involved in the realization of the neurotoxic processes in the spinal cord). Blocking of these receptors inhibits opening of the corresponding calcium channel and to a certain extent may protect neurons of the spinal cord from secondary injury. Ketamine, a noncompetitive blocker of NMDA glutamate receptors, is a promising drug for effective neuroprotective therapy immediately after spinal cord injury. It was proved that ketamine and its metabolite norketamine are high-affinity blockers of NMDA-receptors of the cerebral cortex and spinal cord. There are also studies showing that ketamine also blocks the apoptotic mechanisms in the case of traumatic brain injury [10, 14].

The objective of the study is to assess the experimental model of traumatic spinal cord injury and the efficacy of ketamine for neuroprotection in the complex therapy of acute period of the spinal cord injury.

Material and Methods

The study included 60 rabbits weighing 2500–3400 g with simulated acute spinal cord injury. Standard open spinal cord injury was inflicted in the lower thoracic spine. For this purpose, we performed laminectomy of a lower thoracic vertebra, while the dura was left intact. Traumatic effect was inflicted using the impact device with graduated strength and impact area perpendicular to the spinal cord [4]. All invasive procedure were performed under adequate anesthesia as required by the provisions of the Declaration of Helsinki of the World Medical Association.

Anesthesia during the intervention included intravenous administration of propofol through syringe dispenser at a rate of 16–40 mg/kg/h and intramuscular promedol 7 mg/kg.

After the traumatic impact, complex therapy was conducted (hormonal, antibacterial, infusion). Monitoring included assessment of motor function, reflexes, pelvic organ function, and skin sensitivity.

The experimental animals were divided into several groups: in the control group, acute spinal cord injury was simulated, the wound was sutured in layers, no specific treatment was carried out; in the study group K2, NMDA-receptor blocker therapy (subnarcotic fractional doses of ketamine during 72 hours) was initiated 2 hours after injury; in the study group K16, therapy was initiated 16 hours after the injury.

Spinal cord state was assessed based on 6-point scale of muscular strength [3], evaluation of the tactile and deep sensitivity, pelvic organ function, and trophic disorders.

Results and Discussion

When solving this problem, we developed a model of spinal cord injury, which is reproducible, graduated, same-type, and close to the clinical course of spinal injury. The model enables to master the pathogenetically appropriate complex of measures for prevention and treatment of the consequences of spinal cord injury. The method is easy to use, requires no additional costs, and prevents high mortality of laboratory animals.

The method of simulation of spinal cord injury provides clinical data in rabbits: lower paraparesis, dysfunction of the pelvic organs, symptoms of multifocal lesions of membranes and substance of the spinal cord, corresponding to the level of inflicted injury, which enables quickest possible diagnosis of the injury and immediate administration of medication in order to reduce the risk of complications.

When simulating traumatic injury using this method in animals, severe neurological symptoms in the form of severe lower paresis up to paraplegia, sensory disorders, and pelvic organ dysfunction in the form of retention were observed after termination of anesthetic effects. The condition of all animals was severe, the animals we flaccid, adynamic, had poor appetite, and rapidly lost weight.

When comparing the regression of neurological deficit (motor function recovery) in the control group with that in the group, where ketamine administration was initiated 2 hours after injury, the significance level of the differences was $p = 0.043$ (Pearson's χ^2 test), $p = 0.039$ (χ^2). Therefore, the differences were significant ($p < 0.05$). The groups with later initiation of ketamine therapy demonstrated better motor function recovery, but the differences between these data are not significant ($p > 0.05$). Our results justify the technique of pathophysiological state correction in patients with traumatic spinal cord injury by inclusion of NMDA-receptor blockers in therapy and show that early initiation of the therapy is optimal (Table).

When analyzing the data on other functions (pelvic organ function, superficial and deep sensitivity, trophic disorders), we found differences between the groups, receiving specific therapy, and the control group. However, these differences were not statistically significant ($p > 0.05$).

When evaluating body weight changes over time, we found significant ($p < 0.05$) weight gain in the animals in the group with early inclusion of ket-

Table

Measures of motor activity over time in animals during experimental stages, points (M ± m)

Group	Day 1	Day 7	Day 14
Control	0.600 ± 0.470	0.860 ± 0.360	1.360 ± 0.500
K2	0.640 ± 0.497	1.100 ± 0.267	1.900 ± 0.470*
K16	0.600 ± 0.480	1.000 ± 0.300	1.670 ± 0.490

*p < 0.05 as compared with the control group.

amine therapy as compared to the control group.

Conclusions

1. The proposed model of acute spinal cord injury meets the requirements for the experimental model and can be recommended for studies of therapies of spinal cord injury.

2. Ketamine is an effective neuroprotectant in spinal injury; early administration of this drug significantly improves the results of treatment in the acute period of traumatic spinal cord injury.

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