THE EFFECT OF GABA-BENZODIAZEPINE RECEPTOR COMPLEX MODULATORS (MPTD-01 AND BS 34–20) ON THE CEREBRAL CORTEX AFTER EXPERIMENTAL BRAIN INJURY

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Background. Traumatic brain injury (TBI) is a major medical, social, and public health problem. In the military population the TBI incidence also remains high due to blunt head trauma and blast injuries. Considering the substantial TBI burden on society, including possible posttraumatic epilepsy onset and other comorbidities, the search for new treatment methods, including drug design with the identification of new molecules, is highly relevant.

Aim: The study aimed to evaluate the effect of GABA-benzodiazepine receptor complex modulators (hydrogenated 2,3-benzodiazepines MPTD-01 and BS 34–20) on rat cerebral cortex after traumatic brain injury.

Materials and methods. The experiment was carried out on male Wistar rats. To obtain traumatic brain injury we used the weight drop model. Based on the received treatment, the Rats were divided into intact, placebo (II), and MPTD-01 (III) and BS 34–20 (IV) groups. The cerebral cortex in the impact zone was harvested for examination. The light microscopy was performed on 3, 7, 14, and 21 days after injury.

Results. The observed histological picture of the changes in the cerebral cortex, especially in the II group is similar to those, described by other research papers as mild- to severe traumatic brain injury. The reproduction of the blunt trauma model leads to a series of typical changes that replace each other and can be characterized as alteration, edema, phase of cellular reactions, and repair. Unlike the placebo group, the III and IV experimental groups (GABA-benzodiazepine receptor complex modulators administration) cerebral cortex demonstrated a smoothing of the swelling phase and its reduction, a shift in the time phase of cellular reactions to a later time. In the BS 34–20 group, minimal edema was observed on the 21st day of the experiment.

Conclusion. Such morphological changes can be considered a neuroprotective effect, however, to fully characterize the effects of the GABA-benzodiazepine receptor complex modulators, glial reactions and neuron-glial interactions should be investigated.

Key words: brain injury, GABA receptors, benzodiazepine, drug design.
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**MATERIALS AND METHODS**

The experiment was carried out on 125 male Wistar rats (180–240 g) that were housed in standard conditions, natural light cycle, water, and food ad libitum. We performed blunt traumatic brain injury (TBI) on rats from all experimental groups by using a modified Marmarou weight drop model. 66.7 g weight drop from 65 cm was applied on interauricular point at the sagittal line. Peculiarities of brain injury modeling are well described in previous papers [7].

All of the rats were divided into several groups: rats from the I group rats received no injury or treatment (intact); rats from the II group received placebo treatment; rats from the III group received MPTD-01 (intraperitoneally, in a dose of 5 mg/kg, daily); in the IV group rats received BS 34–20 (intraperitoneally in a dose of 5 mg/kg, daily). 3, 7, 14, and 21 days after injury rats were sacrificed by decapitation under thiopentone overdose. Rat brains were processed for light microscopy. 5-um thick sagittal formalin-fixed paraffin-embedded sections were sliced and stained with hematoxylin and eosyn [8], observed via MICROmed Evolution ES-4130 light microscope. All obtained photos were processed with ImageJ software (NIH, USA, ver. 1.50) We defined and studied the cerebral cortex (impact zone).

All experimental procedures were performed following Directive 2010/63/EU of the European Parliament and of the Council on protection of animals used for scientific purposes. We also consider the national regulatory act (The law of Ukraine “On protection of animals from cruelty”).

![Fig. 1. Impact zone in cerebral cortex in different time after injury; hematoxylin-eosin stain; ×200](image)

A1 – group II 3rd day; B1 – group II, 7th day; C1 – group II, 14th day; D1 – group II, 21st day.
A2 – group III 3rd day; B2 – group III, 7th day; C2 – group III, 14th day; D2 – group III, 21st day.
A3 – group IV 3rd day; B3 – group IV, 7th day; C3 – group IV, 14th day; D3 – group IV, 21st day.
RESULTS AND DISCUSSION

By the 3d day after the injury, rats from all experimental groups demonstrated preserved layer structure of the brain cortex (fig. 1). Along with this, we observe signs of swelling, more pronounced in the II experimental group (TBI+placebo): the molecular layer of the brain cortex preserves its thickness but appears foamy; neurons in external granular and pyramidal layers preserve their shape but demonstrate pericellular edema. Such changes were more prominent in the II (TBI+placebo) group. Such findings were also seen in the III experimental group (TBI+MPTD-01) but the degree of their severity was less than in the II group. Rats from the IV group (TBI+BS 34–20) demonstrated similar but minimal expression changes.

On the 7th day after the injury, rats from the II (TBI+placebo) group demonstrated signs of focal cortical injury: in the brain cortex the external granular layer is not clearly seen, moreover, it contains a narrow impact zone impregnated by erythrocytes that run through all cortical layers. Cells in all cortical layers below the place of impact often have corrugated shape and basophilic cytoplasm. In the III group (TBI+MPTD-01) cortical impact zone contained a foamy focus in the molecular layer and a narrow external granular layer. The majority of the cells in cortical layers below the impact zone have basophilic cytoplasm. In the IV group (TBI+BS 34–20) cortical impact zone appears as a cavity within a foamy transformed molecular layer. Cells of the rest layers preserve their shape, size, orientation, and tinctorial features.

In the III group (TBI+MPTD-01) the cortical impact zone contains foamy debris and a cortical defect zone that spreads down to the pyramidal layer. The majority of the cells in cortical layers below the impact zone have basophilic cytoplasm.

In the IV group (TBI+BS 34–20) the cortical impact zone appears as foamy transformed superficial portion of the molecular layer. The external granular layer contains small neurons with basophilic cytoplasm and pericellular edema. Cells in the rest of the cortical layers preserve their structure and orientation.

By the 21st day in the II group (TBI+placebo) cortical impact zone appears as a foamy portion of the molecular cortical layer. Cells in the rest of the layers preserve their structure and tinctorial properties.

In the III group (TBI+MPTD-01) Impact zone appears as the focus of the narrowing of the cortical molecular layer. Cells of the rest cortical layers demonstrate preserved shape and size but have basophilic cytoplasm.

In the IV group (TBI+BS 34–20) cortical impact appears as a cavity within a foamy transformed molecular layer. Cells of the rest layers preserve their shape, size, orientation, and tinctorial features.

DISCUSSION

At the all terms after TBI the impact affects all of the studied brain structures. At early terms (3 and 7 days after injury) we observe focal foamy neuropil transformation in the molecular cortical layer with highlighting of impact focus 14 days after the injury, in all groups such foamy focus is situated majorly in the molecular layer.

Cells of the brain cortex in layers underlying the impact zone react to impact in the way of increased basophilic cytoplasm straining, which can be considered as a sign of their increased synthetic activity due to injury. However, the timing of such activation is unequal between groups: rats from the II (TBI+placebo) group demonstrate such change in neuronal cytoplasm straining on the 7th day after injury and preserve described changes up to the 21st day. In the III group (TBI+MPTD-01) neuronal cytoplasmatic basophilia appears on the
3rd day after the injury, it also can be observed on the 7th day, but it decreases on the 14th day and neurons restore their tinctorial features until day 21. In the IV group (TBI+BS 34–20) maximum basophilia was observed in time 7–14 days after injury with recovery on the 21st day.

The observed histological picture of the changes in the brain cortex, especially in the II group is similar to those, described by other research papers as mild- to severe traumatic brain injury. [9, 10, 11]

Revealed signs of cerebral cortex edema in all groups can be considered vasogenic due to increased capillary permeability and cellular (cytoxic) due to cytoplasm hyperosmolarity and extracellular hypotonicity [12].

As described above changes in cellular shape and extreme basophilic strain of cytoplasm can indicate either cell activation or early signs of apoptosis.

CONCLUSIONS

Thus, the reproduction of the blunt trauma model leads to a series of typical changes that replace each other and can be characterized as alteration, edema, phase of cellular reactions, and repair.

Unlike the placebo group, the III and IV experimental groups (GABA-benzodiazepine receptor complex modulators administration) demonstrated a smoothing of the swelling phase and its reduction, a shift in the time phase of cellular reactions to a later time. In the BS 34-20 group, minimal edema and more complete restoration of the structure of the hippocampus were observed on the 21st day of the experiment, which can be considered as a neuroprotective effect, however, to fully characterize the effects of the drugs, glial reactions, and neuron-glial interactions should be investigated.

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ВПЛИВ МОДУЛЯТОРІВ ГАМК-БЕНЗОДІАЗЕПІНОВОГО РЕЦЕПТОРНОГО КОМПЛЕКСУ (MPTD-01 ТА BS 34-20) НА КОРУ МОЗКУ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ТРАВМІ ГОЛОВНОГО МОЗКУ

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Актуальність. Черепно-мозкова травма (ЧМТ) є серйозною медичною, соціальною проблемою та проблемою громадського здоров'я. Серед військовослужбовців захворюваність на ЧМТ також залишається високою через тупу травму голови та вибухові травми. Враховуючи значний тягар ЧМТ для суспільства, включно з можливим виникненням посттравматичної епілепсії та іншими супутніми захворюваннями, пошук нових методів лікування, включаючи дизайн препаратів з ідентифікацією нових молекул, є дуже актуальним.

Ціль: оцінити вплив модуляторів ГАМК-бензодіазепінового рецепторного комплексу (гідрогенізованих 2,3-бензодіазепінів MPTD-01 та BS 34-20) на кору головного мозку у щурів після черепно-мозкової травми.

Матеріали та методи. Експеримент проводили на самцях-щурах лінії Вістар. Для отримання ЧМТ використовували модель падіння ваги. Відповідно до отриманого лікування щури були розділені на групи: інтактні (І), плацебо (II), MPTD-01 (III) і BS 34-20 (IV). Кору головного мозку в зоні удару забирали для дослідження. Світлову мікроскопію проводили на 3, 7, 14 і 21 добу після травми.

Результати. Спостережувана гістологічна картина змін кори головного мозку, особливо у ІІ групі, подібна до такої, що описується в інших роботах як ЧМТ легкого та тяжкого ступеня. Відтворення моделі тупої травми призводить до серії типових змін, які змінюють одне одну і можуть бути охарактеризовані як альтерація, набряк, фаза клітинних реакцій і відновлення. На відміну від групи плацебо, у ІІІ та ІV дослідних групах (введення модуляторів комплексу ГАМК-бензодіазепінових рецепторів) у корі головного мозку спостерігалося згладжування фази набряку та її зменшення, зміщення часової фази клітинних реакцій на більш пізній час. У групі BS 34-20 мінімальний набряк спостерігався на 21 добу експерименту.

Висновки. Описані морфологічні зміни попередньо можна вважати нейропротекторним ефектом, однак, щоб повністю охарактеризувати ефекти модуляторів ГАМК-бензодіазепінового рецепторного комплексу, слід дослідити гіліальні реакції та нейрон-гіліальні взаємодії.

Ключові слова: черепно-мозкова травма, ГАМК-рецептори, бензодіазепини, драг-дизайн.