

The Influence of a 2-Benzamido-2-(2-Oxoindolin-3-Iliden) Acetic Acid Derivative on the Behavioral Activity of Rats after Traumatic Brain Injury

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Abstract— We studied the influence of a derivative of 2-benzamido-2-(2-oxoindolin-2-iliden) acetic acid, which is designated as ZNM, on locomotor and orienting–exploratory activity, the state of muscle tonus, and coordination of movements, as well as on the physical stamina of rats after a moderate closed traumatic brain injury. It was revealed that the ZNM derivative promotes a change of the locomotor activity profile with an increase in orienting–exploratory activity and a decrease in the emotionality in animals, an increase in physical stamina in the swimming test with a load, and improved coordination of movements in the rod rotating test. The results of the studies indicate that the pharmacological activity profile of ZNM is similar to that of the reference drug mexidol: cerebroprotective, anxiolytic, and sedative effects.

Keywords: traumatic brain injury, 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative, mexidol, open-field test, rod rotating test, swimming test with load

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Traumatic brain injury (TBI) is considered as the most severe and dangerous pathological state among traumas. TBI remains the main cause of mortality (up to 60% of those traumatized) and disability (up to 25% of such patients) in the age range from 20 to 40. As a rule, the main symptom of closed TBI is muscle-tone disorders, which become apparent in disorders of muscular tone and reflexes, and coordination of complex locomotor acts [1–3].

Previously, while screening studies of 24 derivatives of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid, we demonstrated antihypoxant activity for some of these compounds under conditions of acute hypobaric hypoxia. Among the derivatives we studied, a clear antihypoxant effect was observed for compound no. 15, which was given the name ZNM, making it possible to suggest that this substance has nootropic and neuroprotective properties [4].

The objective of our research was to study the influence of the ZNM 2-benzamido-2-(2-oxoindolin-2-iliden) acetic acid derivative, on the behavioral activity of rats after TBI according to the criteria of locomotor and orientative-exploratory activity, muscle tone and coordination of movements, as well as physical stamina.

MATERIAL AND METHODS

The study was conducted on 32 white non-linear mature male rats with weights from 180 to 200 g, which were divided into four groups ($n = 8$): the first group during TBI modeling was treated with the studied drug, ZNM, at a dose of 15 mg/kg in the form of an aqueous suspension stabilized with polysorbate 80 (Twin 80); the second group with TBI was treated via administration of the reference drug mexidol at a dose of 100 mg/kg; the third (control group) received the equivalent amount of solvent; the fourth group was the intact control (ether anesthesia without TBI). A moderate TBI model was reproduced under ether semiarousness via an injury to the parietooccipital head area with a 0.0495 kg load with an energy of 0.015 J [5]. The drugs were administered in the prophylactic and curative regimen for 3 days before the TBI (the last injection was made 30 minutes before TBI) and 2 days after it. In the acute period of the trauma (48 h after TBI modeling), using the open-field test we evaluated the behavioral and neural disorders, evaluated locomotor activity (the number of crossed squares, the number movements into the center), orienting–exploratory activity (the number of standing incidents and explored holes), and emotional reactions (the number of groomings, defecations, and urinations). Physical stamina was evaluated in the swimming test with a 20% body mass load; coordination of movements and

Table 1. The influence of ZNM and mexidol on behavioral and emotional reactions in the open-field test in rats 48 h after modeling of the moderate traumatic brain injury ($M \pm m$), ($n = 8$).

Indices (for 5 min)	Intact control	Traumatic brain injury		
		control pathology	ZNM	mexidol
Locomotor activity				
Crossed squares	25.60 ± 2.5	15.37 ± 4.21	10.48 ± 3.27	18.43 ± 4.90
Movements into the center	4.00 ± 0.33	1.83 ± 0.13	3.54 ± 0.20	1.90 ± 0.47
Sum	29.6 ± 2.67	17.2 ± 4.30*	14.02 ± 3.30*	20.33 ± 4.95* [^]
Orienting—exploratory activity				
Stances	7.40 ± 1.20	3.00 ± 0.74	4.20 ± 0.46	5.31 ± 1.38
Holes	6.10 ± 1.30	4.00 ± 1.32	4.37 ± 1.66	4.81 ± 1.25
Sum	13.50 ± 1.7	7.00 ± 1.93*	7.57 ± 1.84*	10.12 ± 1.50*
Emotional reactions				
Grooming	4.40 ± 0.46	8.20 ± 0.87	2.67 ± 0.31	3.88 ± 0.82
Boluses	2.20 ± 0.31	5.63 ± 1.69	1.50 ± 0.72	3.10 ± 0.94
Urinations	1.76 ± 0.27	3.42 ± 0.42	1.22 ± 0.21	2.45 ± 0.73
Sum	8.36 ± 0.68	17.25 ± 1.90*	5.39 ± 1.47* [#]	9.43 ± 1.20* ^{#^}

Comments: *, the index of significance in comparison with the control data; #, the index of significance in comparison with data of TBI group; ^, the index of significance in comparison with data of ZNM group.

muscle tonus were evaluated by a test with a rod that rotated at ten revolutions per minute [6].

The animals were kept under standard vivarium conditions, under constant temperature and air humidity, with free access to water and food. All manipulations were conducted according to Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

The statistical analysis of the obtained results was conducted using SPSS Statistics 17.0 software via the Student's *t*-test, White's test, and *F*-test.

RESULTS AND DISCUSSION

As a result of these studies, it was noted that in the first group of animals (administration of ZNM on the background of traumatic brain injury) the locomotor activity decreased by 1.2 times due to a decrease in the number of crossed squares; however, the number of movements into the open-field center increased; the orienting—exploratory activity increased by 1.1 times. The indices of emotional reactions and their vegetative accompaniment decreased due to defecations and urinations by 3.2 times compared to the control pathology group ($p < 0.05$). These data are the evidence of the change in the locomotor activity profile of animals with an increase in their orienting—exploratory activ-

ity and a decrease in their emotionality after TBI against the background of ZNM application (Table 1).

In the rotating rod test, the indices of muscle tone and coordination of movements in the ZNM group and the reference drug (mexidol) group were the same: number of animals that fell before 30 s was two (25%); 30 s to 1 min—three (37.5%); later than 1 min—three (37.5%), which significantly exceeded the indices of the intact control ($p < 0.05$) (Table 2).

Thus, based on the results of the study using a moderate closed TBI model, ZNM, a 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative led to a change of the locomotor activity profile of the animals with an increase of their orientating—exploratory activity and decrease of their emotionality in the open field test, increased physical stamina in the swimming test with a load and improved the coordination of movements in the rotating-rod test. The results of these studies indicate the similar pharmacological activity profile of ZNM and that of the reference drug mexidol: cerebroprotective, anxiolytic, and sedative effects.

CONCLUSIONS

A 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative, ZNM, ameliorates the manifestations

Table 2. The influence of ZNM and mexidol on the coordination of movements and physical stamina 48 h after modeling of moderate traumatic brain injury ($M \pm m$), ($n = 8$)

Indices (for 5 min)	Intact control	Traumatic brain injury		
		control pathology	ZNM	mexidol
Muscle tone and coordination of movements (rotating-rod test)				
Number of animals that fell				
Before 30 s	1	6	2	2
From 30 s to 1 min	2	1	3	3
After 1 min	3	1	3	3
Physical stamina (forced swimming with a load)				
Duration of swimming	42.10 ± 2.48	28.40 ± 3.22	39.30 ± 2.68	40.40 ± 3.21

of traumatic brain injury and recovers the behavioral activities of test animals.

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