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EFFECT OF THE DERIVATIVE OF 2-BENZAMIDO-2-(2-OXOINDOLIN-3-ILIDEN) ACETIC ACID ON THE ACTIVITY OF FREE-RADICAL PROCESSES IN TRAUMATIC BRAIN INJURY

In experiments on adult laboratory rats the effect of the derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid under the symbol ZNM on the activity of the lipid and protein peroxidation and antioxidant system in blood plasma and brain, as well as on the activity of the key enzyme of energy metabolism succinate dehydrogenase in the brain of animals with a closed traumatic brain injury (TBI) of moderate severity in comparison with the reference drug mexidol was investigated. It was established that ZNM not significantly concedes to the action of mexidol in its cerebroprotective on antioxidant properties under the conditions of TBI, contributing to normalization of the prooxidant-antioxidant balance in the blood plasma and brain of rats, as well as improving the energy metabolism in the cells of the central nervous system.

Key words: derivatives of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid; mexidol; traumatic brain injury; lipid and protein peroxidation; antioxidant system; energy metabolism

INTRODUCTION

Traumatic brain injury (TBI) is the most severe and serious pathological condition in the structure of traumatism. TBI continues to be the main cause of death (up to 60 % among injured) and disability of population (25 % of survived patients) in the age group of 20-40 years. Acute period of traumatic disease (TD) is a typical pathological process, called posttraumatic endogenous intoxication (traumatic toxicosis). In TBI brain is the main source of endotoxemia in the body [3, 4]. One of the main pathogenesis factors in acute period of TD in TBI is cerebral hypoxia, which leads to progressive brain tissue acidosis, intracellular edema and depletion of the macroergic compounds pool [10]. In its turn, the processes of an intensification of cell membranes damage initiates, primarily – by lipid peroxidation (LPO), free radical destruction of proteins, inactivation of enzymes and microcirculatory disorders [9].

During the screening studies of the 24 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivatives an antihypoxic activity of certain compounds under the conditions of acute hypobaric hypoxia was established [11]. Among the studied derivatives the most significant antihypoxic activity was observed when using compound number 15 (ZNM) [11, 12], which suggests the neuroprotective properties of this substance.

The aim of the study was to establish the impact of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative ZNM on the activity of free radical processes in rats TBI.

MATERIALS AND METHODS

The research was conducted on 32 white nonlinear mature male rats weighting 180-200 g, divided into 4 groups (n = 8): the first group was injected intraperitoneally the substance ZNM at a dose of 15 mg/kg in the form of an aqueous suspension stabilized by polysorbate 80 (Tween 80) prior the TBI of moderate severity modeling; the second group was administered prior the TBI the reference drug mexidol at a dose of 100 mg/kg; the third (control) group was administered an equivalent amount of solvent; the fourth group – intact control (ether anesthesia without TBI). TBI of moderate severity was modeled under the ether anesthesia with a standardized weight-drop device (0.0495 kg, 0.315 J) inducing a focal blunt injury over the unprotected parietal-occipital head area [2]. Drugs were administered in prophylactic and therapeutic regimen 3 days before (last – 30 minutes prior TBI) and 2 days after it, after which the animals were decapitated under the light ether anesthesia. The animals were kept under the standard vivarium conditions at a constant temperature and humidity with free access to food and water. All manipulations were carried out in accordance with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

Table 1

INFLUENCE OF THE 2-BENZAMIDE-2-(2-OXOINDOLIN-3-ILIDEN) ACETIC ACID DERIVATIVE ZNM ON THE LEVEL OF LIPID AND PROTEIN PEROXIDATION PRODUCTS AND ACTIVITY OF THE ANTIOXIDANT SYSTEM IN BLOOD OF RATS WITH A CLOSED TRAUMATIC BRAIN INJURY (M ± m, n = 8)

Index	Control	Model pathology (TBI)	TBI + ZNM	TBI + Mexidol
MDA level, μmol/l	9.17 ± 0.45	19.92 ± 0.56 $p_1 \le 0.01$	11.79 ± 0.27 $p_1 \le 0.01$ $p_2 \le 0.01$	15.4 ± 1.82 $p_1 \le 0.01$ $p_2 \le 0.05$ $p_3 > 0.05$
OMP level, o.d.u./ml	0.82 ± 0.02	0.91 ± 0.01 $p_1 \le 0.05$	0.86 ± 0.03 $p_1 > 0.05$ $p_2 \le 0.05$	0.98 ± 0.08 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$
CAT activity, μmol/(min×l)	24.76 ± 1.05	9.62 ± 0.14 $p_1 \le 0.01$	12.70 ± 0.28 $p_1 \le 0.01$ $p_2 \le 0.01$	11.95 ± 0.23 $p_1 \le 0.01$ $p_2 > 0.05$ $p_3 > 0.05$
CP level, mg/l	156.3 ± 2.73	176.4 ± 1.17 $p_1 > 0.05$	141.9 ± 7.77 $p_1 > 0.05$ $p_2 \le 0.05$	150.7 ± 4.34 $p_1 > 0.05$ $p_2 \le 0.01$ $p_3 > 0.05$
SH-groups level, μmol/ml	4.33 ± 0.02	1.64 ± 0.06 $p_1 \le 0.01$	2.7 ± 0.04 $p_1 \le 0.01$ $p_2 \le 0.01$	3.4 ± 0.23 $p_1 \le 0.01$ $p_2 \le 0.01$ $p_3 > 0.05$

Note: p_1 – index of significance comparing to control; p_2 – index of significance comparing to model pathology; p_3 – index of significance comparing to substance ZNM; o.d.u. – optical density units.

To study the free radical processes the plasma and homogenates of the animals' brain were used. Activity of lipid peroxidation was evaluated by the content of malone dialdehyde (MDA), determined by the reaction with 2-thiobarbituric acid and protein peroxidation – by the level of oxidatively modified proteins (OMB), determined by reaction with 2,4-dynitrofenylhydrazine to form hydrazones of the characteristic absorption spectrum [1]. Antioxidant system (AOS) was evaluated by the activity of catalase (CAT) in the reaction with ammonium molybdate, ceruloplasmin level (CP) by the oxidation reaction of phenylenediamine and SH-groups level [7]. The degree of the cellular energy metabolism disturbances in brain was evaluated by the activity of succinate dehydrogenase (SDH) [5]. Statistical analysis of the results was performed using SPSS Statistics 17.0 and Microsoft Excel 2013. Statistical significance was evaluated using parametric Student's t-test (for normal distribution) and non-parametric Mann-Whitney U-test (in case of non-normal distribution). The critical level of significance was accepted with $p \le 0.05$.

RESULTS AND DISCUSSION

It was experimentally established that TBI is accompanied by a decrease of the antioxidant brain defense, manifested by the deficiency of antioxidant enzymes and non-enzymatic components of AOS (Tab. 1, 2). Thus, in the model pathology (TBI) group a decreased activity of CAT by 2.6 times in plasma, and by 1.6 times – in the brain homogenates was registered. It was accompanied by the increased level of lipid peroxidation product MDA in blood plasma by 2.2 times and in brain homogenates – by 1.5 times; and an analogous increase of protein peroxidation products (OMB) level both in blood plasma (by 2.2 times) and in brain homogenates (by 1.6 times). Blood level of SH-groups decreased by 2.6 times. The activity of succinate dehydrogenase (SDH) in brain homogenates was decreased by 8.5 times, indicating the significant disturbance of aerobic metabolism in the central nervous system cells, corresponding to expected changes by the literature data [3, 6, 8, 9].

However, in the group of rats administered with the substance ZNM, a normalization of free radical oxidation of macromolecules and AOS activity after TBI was observed. Use of ZNM significantly reduced the level of MDA both in plasma and in brain structures (by 40.8 % and 17.1 % respectively) and OMB level to the control indices. CP content in plasma decreased by 19.6 % and fit the control level, the content of SH-groups increased by 1.6 times. CAT activity increased in blood plasma and didn't differ significantly from that of control in brain homogenates. Under the influence of ZNM the SDH activity in brain structures increased by 2.5 times.

Thus, the substance ZNM with antihypoxic activity [11, 12] normalizes the state of prooxidant-antioxidant balance in the brain structures and in the whole organism of animals with TBI and improves energy metabolism in the cells of central nervous system. This suggests that the derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid ZNM has antioxidant and cerebroprotective properties.

Table 2

INFLUENCE OF THE 2-BENZAMIDE-2-(2-OXOINDOLIN-3-ILIDEN) ACETIC ACID DERIVATIVE ZNM ON THE LEVEL OF LIPID AND PROTEIN PEROXIDATION PRODUCTS, CATALASE AND SUCCINATE DEHYDROGENASE ACTIVITY IN BRAIN OF RATS WITH A CLOSED TRAUMATIC BRAIN INJURY (M ± m, n = 8)

Index	Control	Model pathology (TBI)	TBI + ZNM	TBI + Mexidol
MDA level, μmol/g of protein	24.3 ± 0.13	35.7 ± 0.69 $p_1 \le 0.05$	29.6 ± 0.68 $p_1 \le 0.05$ $p_2 \le 0.01$	21.9 ± 0.64 $p_1 > 0.05$ $p_2 \le 0.01$ $p_3 \le 0.05$
OMP level, o.d.u./g of protein	11.23 ± 0.46	17.73 ± 0.34 $p_1 \le 0.05$	12.87 ± 1.87 $p_1 > 0.05$ $p_2 \le 0.05$	15.84 ± 0.64 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$
CAT activity, μmol/(min×mg of protein)	10.05 ± 2.50	6.30 ± 0.93 $p_1 \le 0.01$	8.54 ± 0.90 $p_1 > 0.05$ $p_2 > 0.05$	7.32 ± 0.64 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$
SDH activity, nmol/(min×mg of protein)	9.67 ± 0.43	1.14 ± 0.14 $p_1 \le 0.01$	2.85 ± 0.19 $p_1 \le 0.01$ $p_2 \le 0.01$	3.15 ± 0.26 $p_1 \le 0.01$ $p_2 \le 0.01$ $p_3 > 0.05$

Note: The symbols are the same as in the Tab. 1.

The action of substance ZNM coincides to the effect of reference drug mexidol. Although the substance ZNM slightly concedes to antioxidant effect of mexidol in reduction of the lipid peroxidation in brain cells (Tab. 2), but exceeds the effect of mexidol in normalization of protein peroxidation in the cells of brain and MDA level in blood plasma. Concerning other investigated parameters in blood plasma and brain structures, any of significant difference between the actions of ZNM and mexidol wasn't revealed (Tab. 1, 2).

CONCLUSIONS

1. The derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid ZNM exhibits antioxidant and cerebroprotective properties under the conditions of closed brain injury of moderate severity, contributing to normalization of the prooxidant-antioxidant balance in plasma and brain of rats and improving energy metabolism in cells of the central nervous system.
2. The derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid ZNM doesn't concede significantly to the effect of reference drug mexidol under the conditions of a closed traumatic brain injury of moderate severity in the normalization of energy metabolism in nerve cells of rats and prooxidant-antioxidant balance in plasma and brain.

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ВПЛИВ ПОХІДНОГО 2-БЕНЗАМІДО-2-(2-ОКСОІНДОЛІН-3-ІЛІДЕН) ОЦТОВОЇ КИСЛОТИ НА АКТИВНІСТЬ ВІЛЬНОРАДИКАЛЬНИХ ПРОЦЕСІВ ПРИ ЧЕРЕПНОМОЗКОВІЙ ТРАВМІ

В експериментах на статевозрілих лабораторних щурах досліджено дію похідного 2-бензамідо-2(2-оксоіндолін-3-іліден) оцтової кислоти під умовною назвою ZNM на активність вільнорадикального окиснення ліпідів і білків та стан антиоксидантної системи в плазмі крові та головному мозку, а також активність ферменту енергетичного обміну сукцинатдегідрогенази в головному мозку при закритій черепномозковій травмі (ЧМТ) середньої тяжкості у порівнянні з антигіпоксантом мексидолом. Встановлено, що ZNM суттєво не поступається дії мексидолу при ЧМТ щодо антиоксидантних і церебропротекторних властивостей, сприяючи нормалізації прооксидантно-антиоксидантного балансу в плазмі крові та головному мозку щурів, а також покращуючи енергетичний обмін у клітинах центральної нервової системи.

Ключові слова: похідне 2-бензамідо-2(2-оксоіндолін-3-іліден) оцтової кислоти; мексидол; черепномозкова травма; ліпідна і білкова пероксидація; антиоксидантна система; енергетичний обмін

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ВЛИЯНИЕ ПРОИЗВОДНОГО 2-БЕНЗАМИД-2(2-ОКСОИНДОЛИН-3-ИЛИДЕН) УКСУСНОЙ КИСЛОТЫ НА АКТИВНОСТЬ СВОБОДНОРАДИКАЛЬНЫХ ПРОЦЕССОВ ПРИ ЧЕРЕПНОМОЗГОВОЙ ТРАВМЕ

В экспериментах на половозрелых лабораторных крысах исследовано действие производного 2-бензамид-2(2-оксоиндолин-3-илиден) уксусной кислоты под условным обозначением ZNM на активность липидной и белковой пероксидации и состояние антиоксидантной системы в плазме крови и головном мозге, а также активность фермента энергетического обмена сукцинатдегидрогеназы в головном мозге при закрытой черепномозговой травме (ЧМТ) средней тяжести по сравнению с антигипоксантом мексидолом. Установлено, что ZNM существенно не уступает действию мексидола при ЧМТ по антиоксидантным и церебропротекторным свойствам, способствуя нормализации прооксидантно-антиоксидантного баланса в плазме крови и головном мозге крыс, а также улучшая энергетический обмен в клетках центральной нервной системы.

Ключевые слова: производное 2-бензамид-2(2-оксоиндолин-3-илиден) уксусной кислоты; мексидол; черепномозговая травма; липидная и белковая пероксидация; антиоксидантная система; энергетический обмен

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