**The effect of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative on the activity of lipid peroxidation processes and the antioxidant defense in chronic hypobaric hypoxia**

Hypoxia is a typical pathological process developing due to the decreased concentration of oxygen in the organism or in the particular tissues and organs, as well as disturbances of oxygen utilization during biological oxidation.

**Aim.** To determine the antihypoxic and antioxidant activity of the ZNM derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid in the brain and blood plasma of rats under the conditions of chronic hypobaric hypoxia (CHH) by the indicators of the lipid peroxidation processes activity and the state of the antioxidant defense.

**Materials and methods.** The studies were conducted under the conditions of CHH modeled by the standard method. The ZNM substance in the dose of 15 mg/kg and the reference drug mexidol in the dose of 100 mg/kg were injected intraperitoneally starting from the 14th day of the experiment 30 min prior to the hypoxia simulation.

**Results.** In the group of animals treated with ZNM a significant decrease in the content of malondialdehyde in the blood plasma and brain structures (by 1.2 and 1.1 times, respectively), as well as in the content of oxidative modified proteins (by 1.2 times in the blood plasma and 1.4 times in the brain tissue) was observed. The content of ceruloplasmin in the blood plasma decreased by 1.7 times; the content of SH-groups increased by 3.1 times; the catalase activity increased by 1.2 times in the blood plasma, and by 1.9 times in brain homogenates. The reduced glutathione level in brain homogenates decreased by 1.7 times; the content of oxidized glutathione increased by 2.1 times in the blood plasma and by 1.3 times in brain homogenates compared to the model pathology group.

**Conclusions.** It has been found that both mexidol and the ZNM substance demonstrate a significant antioxidant activity under the conditions of CHH by stabilizing the balance between prooxidant and antioxidant systems of the body in rats, and it is particularly important in oxidative stress caused by chronic hypoxia.

**Key words:** antihypoxants; chronic hypobaric hypoxia; 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid derivative; mexidol
Влияние производных 2-бензамидо-2(2-оксоиндолин-3-илiden) уксусной кислоты на активность процессов липопероксидации и состояние антиоксидантной защиты при хронической гипобарической гипоксии

Цель. Целью исследования стало установление антигипоксантной и антиоксидантной активности произвольного 2-бензамидо-2(2-оксоиндолин-3-илiden) уксусной кислоты ZNM в головном мозге и плазме крови крыс в условиях хронической гипобарической гипоксии (ХГГ) по показателям активности процессов липопероксидации и состояния антиоксидантной защиты у крыс.

Материалы и методы. Исследования проводились в условиях ХГГ, которую моделировали по стандартной методике. Вещество ZNM в дозе 15 мг/кг и препарат сравнения мексидол в дозе 100 мг/кг вводили внутрибрюшинно, начиная с 14 суток моделирования гипоксии за 30 мин до начала сеанса.

Результаты. В группе животных, которым вводили исследуемое вещество ZNM, наблюдали достоверное снижение содержания малонового диальдегида как в плазме крови, так и в структурах головного мозга (соответственно в 1,2 раза и в 1,1 раза), а также содержание продуктов окислительной модификации белков (для плазмы крови — в 1,2 раза, а для ткани головного мозга — в 1,4 раза). Содержание церулоплазмина в плазме крови уменьшилось в 1,7 раза, а содержание SH-групп увеличилось в 3,1 раза. При этом активность каталазы в плазме крови повысилась в 1,2 раза, а в гомогенатах головного мозга — в 1,9 раза. Содержание глутатиона восстановленного в гомогенатах головного мозга повысилось в 1,4 раза, а активность глутатионпероксидазы повысилась в плазме крови в 2,1 раза, а в гомогенатах — в 1,3 раза.

Выводы. Итак, установлено, что вещество ZNM существенно не уступает действию мексидола при ХГГ по антигипоксантным и антиоксидантным свойствам, а также способствует нормализации прооксидантно-антиоксидантного баланса в плазме крови и головном мозге крыс.

Ключевые слова: антигипоксанты; хроническая гипобарическая гипоксия; производные 2-бензамидо-2(2-оксоиндолин-3-илiden) уксусной кислоты; мексидол.
30 min prior to the hypoxia simulation. At the end of the 4th week animals were decapitated under the light ether anesthesia.

To study the free radical processes the plasma and brain homogenates of animals were used. The activity of lipid peroxidation was assessed by the content of malondialdehyde (MDA) determined by the reaction with 2-thiobarbituric acid, and protein peroxidation – by the content of oxidative modified proteins (OMP) determined by the reaction with 2,4-dinitrofenylhydrazine to form hydrazones of the characteristic absorption spectrum. The state of the antioxidant system (AOS) was assessed by the activity of catalase (CAT) in the reaction with ammonium molybdate, by the ceruloplasmin level (CP) determined by the oxidation reaction of phenylendiamine and by the content of SH-groups. The glutathione peroxidase (GPx) activity was assessed by the amount of reduced glutathione (G-SH) which was not used in the enzymatic reaction. The G-SH level was determined in the reaction with potassium iodide [10, 11].

Statistical analysis of the results was performed using SPSS Statistics 17.0 and Microsoft Excel 2013. The statistical significance was assessed 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 as p≤0.05.

**Results and Discussion**

The results of the experiments conducted (Tab. 1 and 2) demonstrated that there was intensification of free radical processes along with reduction of the activity of both enzymatic and non-enzymatic components of the antiradical defense in the model pathology group. Particularly, in the blood plasma the MDA level increased by 1.3 times, the OMP level – by 1.5 times, and the CAT activity significantly decreased by 1.5 times compared to the intact animals. The CP level compensatorily increased by 1.9 times with simultaneous decrease the content of SH-groups by 3.7 times, and GPx – by 2.2 times. In addition, the CAT activity reduced by 2.2 times in brain homogenates, the GPx activity – by 1.3 times, and the content of G-SH – by 1.5 times. The content of lipid and protein peroxidation products increased by 1.3 times compared to the intact control.

In the group of animals treated with ZNM the normalization of free radical oxidation of macromolecules and the AOS activity was observed. The use of ZNM significantly decreased the MDA level and non-parametric Mann-Whitney U-test (in case of non-normal distribution). The critical level of significance was accepted as p≤0.05.

### Table 1

The effect of the ZNM derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid on the content of lipid and protein peroxidation products and the activity of the antioxidant system in the blood plasma of rats with chronic hypobaric hypoxia (M±m, n=8)

| Index        | Control          | Model pathology (CHH) | CHH + ZNM | CHH + mexidol |
|--------------|------------------|-----------------------|-----------|---------------|
| MDA, µmol/l  | 12.34±0.65       | 15.78±0.46            | 13.36±3.17 | 12.72±2.52    |
| OMP, o.d.u./ml| 0.78±0.07        | 1.16±0.02             | 0.93±0.05  | 0.82±0.08     |
| CAT, µmol/(min×l) | 14.46±1.65     | 9.92±0.64             | 12.27±0.48 | 13.65±0.53    |
| GPx, nmol/(min×l) | 113.43±1.13    | 50.71±3.60            | 105.47±2.82 | 109.87±0.31   |
| CP, mg/l     | 166.2±2.43       | 320.7±2.30            | 185.4±4.27 | 173.6±4.44    |
| SH-groups, µmol/ml | 4.58±0.03      | 1.24±0.02             | 3.83±0.07  | 4.22±0.23     |

Note. * – the index of significance compared to the intact control; # – the index of significance compared to the model pathology; > – the index of significance compared to the ZNM substance; o.d.u. – optical density units; MDA – malondialdehyde; OMB – oxidative modified proteins; CAT – catalase; GPx – glutathione peroxidase; CP – ceruloplasmin; CHH – chronic hypobaric hypoxia.

### Table 2

The effect of the ZNM derivative of 2-benzamido-2-(2-oxoindolin-3-iliden) acetic acid on the content of lipid and protein peroxidation products and the activity of the antioxidant system in the brain of rats with chronic hypobaric hypoxia (M±m, n=8)

| Index        | Control          | Model pathology (CHH) | CHH + ZNM | CHH + mexidol |
|--------------|------------------|-----------------------|-----------|---------------|
| MDA, µmol/g  | 33.4±1.60        | 43.3±1.5              | 37.8±1.20  | 35.32±1.73    |
| G-SH, µmol/g | 4.25±0.02        | 2.83±0.01             | 3.97±0.05  | 4.07±0.09     |
| OMP, o.d.u/g | 10.83±0.45       | 14.53±0.62            | 12.17±1.35 | 11.69±2.04    |
| CAT, µmol/(min×mg) | 3.85±0.50   | 1.76±0.69             | 3.27±0.50  | 3.42±0.54     |
| GPx, nmol/(min×mg) | 170.4±3.40   | 130.9±5.66            | 164.23±3.52 | 167.14±3.78   |

Note. G-SH – reduced glutathione; other symbols are the same as in Table 1.
in the blood plasma and brain structures (by 1.2 and 1.1 times, respectively), the OMP level (by 1.2 times in the blood plasma and by 1.4 times in the brain tissue). The CP content in the blood plasma had 1.7 times decrease, and the content of SH-groups increased by 3.1 times. The CAT activity in the blood plasma increased by 1.2 times in the blood plasma, and in brain homogenates – by 1.9 times. The G-SH level in brain homogenates increased by 1.4 times, the GPx activity significantly increased by 2.1 times in the blood plasma and by 1.3 times in brain homogenates compared to the model pathology group.

The action of the ZNM substance corresponds to the action of the antihypoxant reference drug mexidol by the effect on the prooxidant-antioxidant indices studied in the blood plasma and in brain structures although somewhat inferior to it.

In general, the ZNM substance prevents negative shifts of the prooxidant-antioxidant balance in the organism of rats affected by CCH.

Therefore, both mexidol and the ZNM substance demonstrate a significant antioxidant activity under the conditions of CHH by stabilizing the balance between prooxidant and antioxidant systems of the body in rats, and it is particularly important in oxidative stress caused by chronic hypoxia.

CONCLUSIONS

The ZNM derivative of 2-benzamido-2-(2-oxoindolin-3-ylidene)acetic acid exhibits the antioxidant properties under the conditions of chronic hypobaric hypoxia and promotes normalization of the prooxidant-antioxidant balance in the blood plasma and the brain of rats.

Conflicts of Interest: authors have no conflict of interest to declare.

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Заморський І. І., доктор медицинских наук, профессор, завідувач кафедри фармакології, Вищий державний навчальний заклад України «Буковинський державний медичний університет»

Заморский И. И., доктор медицинских наук, профессор, заведующий кафедрой фармакологии, Вышее государственное учебное заведение Украины «Буковинский государственный медицинский университет»

Букатару Ю. С., ассистент кафедры фармакології, Вищий державний навчальний заклад України «Буковинський державний медичний університет»

Букатару Ю. С., ассистент кафедры фармакологии, Высшее государственное учебное заведение Украины «Буковинский государственный медицинский университет»

Колісник С. В., доктор фармацевтичних наук, професор кафедри аналітичної хімії, Національний фармацевтичний університет

Колесник С. В., доктор фармацевтических наук, профессор кафедры аналитической химии, Национальный фармацевтический университет

Mailing address: 2, Theatralna sq., Chernivtsi, 58002, Pharmacology Department, Higher State Educational Institution of Ukraine «Bukovinian State Medical University», E-mail: zamorskii@mail.ru

Адреса для переписки: 58002, г. Черновцы, Театральная пл., 2, кафедра фармакологии ВДНУ Украины «Буковинский державний медичний університет». E-mail: zamorskii@mail.ru

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