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THE INVESTIGATION OF ORGANOPROTECTIVE PROPERTIES OF 2-HYDROXY-N-NAPHTHALENE-1-YL -2-(2-OXY-1,2-DIHYDRO-INDOLE-3-YLIDEN)-ACETAMIDE

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In experiments on 64 white male rats, the effect of 2-hydroxy-n-naphthalene-1-yl-2-(2-oxy-1,2-dihydro-indole-3-yliden)-acetamide (12 mg/kg) compared to ethylmethylhydroxypyridine succinate (50 mg/ml) on the course of acute kidney injury caused by glycerol and tetrachloromethane hepatitis was studied. It has been found that the use of 2-hydroxy-n-naphthalene-1-yl-2-(2-oxy-1,2-dihydro-indole-3-yliden)-acetamide against the background of tetrachloromethane hepatitis exhibits hepatoprotective properties, as evidenced by a decrease in the organ mass coefficient and the activity of enzymes-markers of damage to hepatocytes of alanine aminotransferase and aspartate aminotransferase in blood serum. The hepatoprotective effect of the studied compound is not inferior to ethylmethylhydroxypyridine succinate. In addition, a derivative of 2-oxyindoline-3-glyoxylic acid reduced the onset time of thiopental anesthesia. In acute glycerol-induced kidney injury, 2-hydroxy-n-naphthalene-1-yl-2-(2-oxy-1,2-dihydro-indole-3-yliden)-acetamide had only the antiproteinuric action and did not show sufficient protective effect. This indicates the absence of the nephroprotective effect in the substance and possible side effects from the kidneys.

Key words: 2-oxyindoline-3-glyoxylic acid derivative, liver, kidneys, tetrachloromethane, glycerol, rats.

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ДОСЛІДЖЕННЯ ОРГАНОПРОТЕКТОРНИХ ВЛАСТИВОСТЕЙ 2-ГІДРОКСИ-N-НАФТАЛЕН-1-ІЛ-2-(2-ОКСО-1,2-ДИГІДРО-ІНДОЛ-3-ІЛІДЕН)-АЦЕТАМІДУ

У дослідах на 64 білих щурах самцях вивчали вплив 2-гідрокси-n-нафтален-1-іл-2-(2-оксо-1,2-дигідро-індол-3-іліден)-ацетаміду (12 мг/кг) у порівнянні з етилметилгідроксипіридину сукцинатом (50 мг/мл) на перебіг гострого ураження нирок, індукованого гліцеролом та тетрахлорметанового гепатиту. Встановлено, що застосування 2-гідрокси-n-нафтален-1-іл-2-(2-оксо-1,2-дигідро-індол-3-іліден)-ацетамід на тлі тетрахлорметанового гепатиту виявляє гепатопротекторні властивості, про що свідчило зменшення коефіцієнту маси органу і активності ферментів-маркерів ушкодження гепатоцитів аланінамінотрансферази й аспартатамінотрансферази у сироватці крові. За гепатопротекторною дією сполука, що досліджується не поступається етилметилгідроксипіридину сукцинату. Також похідне 2-оксоіндолін-3-гліоксилової кислоти скорочувало час настання тіопенталового наркозу. При гострому гліцерол-індукованому ураженні нирок 2-гідрокси-n-нафтален-1-іл-2-(2-оксо-1,2-дигідро-індол-3-іліден)-ацетамід чинив лише антипротеїнурічну дію й не виявляв достатнього захисного впливу. Це свідчить про відсутність нефропротекторної дії у речовини та про можливі побічні ефекти з боку нирок.

Ключові слова: похідне 2-оксоіндолін-3-гліоксилової кислоти, печінка, нирки, тетрахлорметан, гліцерол, щури.

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According to the WHO, over 300 million people suffer from depression and it equals to 4.4 % of population [15]. Recent investigations have detected that among patients with chronic renal disease, who are not at kidney dialysis, depression spread occurs in 3 times at higher level, than in general population. During end-stage kidney disease, it occurs in 14–30 % of cases [10]. In patients with chronic renal disease along with end-stage kidney disease, depression is a factor, which causes life quality decrease and increase of mortality [14]. It should be noted, that to 85 % of chronic diseases of digestive system are accompanied with emotional disorders and in particular depressive conditions, which require correction [7]. Considering above mentioned it should be necessary to search for anti-depressive drugs which are effective and safe during comorbide pathology of inner organs.

Derivatives of 2-oxyindoline-3-glyoxylic acid, in particular, 2-hydroxy- N-naphthalene-1-yl-2-(2-oxy-1,2-dihydro-indole-3-yliden)-acetamide (substance 18) are important, which have wide spectrum of neurotropic and antihypoxic action which was proved in the experiment [5. 12].

The purpose of the study was to investigate the influence of substance 18 on the spread of acute kidney injury, induced by glycerol and tetrachlorolnethane hepatitis in the experiment.

Materials and methods. Experiments were carried out on 64 sexually mature white rats with body weight 270–310 grams with bioethical principles and norms of Directive of the European Parliament and of the Council 2010/63/EU "About animals' protection, which are used with scientific aim" [13]. During the experiment, animals were held in vivarium with temperature regimen and air humidity on standard regimen with free access to water and food [6].

The investigated substance 2-hydroxy-N-naphthalene-1-yl-2-(2-oxy-1,2-dihydro-indole-3-yliden) with name (substance 18) was synthesized and standardized on the Department of Analytical Chemistry of National Pharmaceutical University (Kharkiv) by PharmDr, Professor S.V. Kolisnyk. Comparison preparation presents ethylmethylhydroxypyridine succinate ("Armadyne", solution for injection, 50 mg/ml, "Lekkhim Kharkiv", Ukraine), as drug with antioxidant, antihypoxic, neuroprotective properties, which is familiar to its pharmacological profile with investigated substance [3].

Substance 18 and reference drug was introduced intraperitonealy (IP) during prevention once a day during 3 days: substance 18 in 12 mg/kg (a dosage was established in previous studies) [11], "Armadyne" – in 100 mg/kg [1]. Intact animals and animals of control group received IP 0.9 % sodium chloride.

Hepatoprotective action of substance 18 (12 mg/kg) was studied on the model of acute tetrachlorolnethane hepatitis [1, 6]. Toxic agent was administered in 50 % of oil solution in 0.8 ml/100 g of mass body subcutaneously during 2 days. Rats were involved in orthothanasia by thiopental narcosis (50 mg/kg) by blood test to its arrest. Survival of animals in group (%), coefficient of liver mass, time of thiopental narcosis, in blood serum hepatocytes' activity of enzymes: alanine-aminotransferase ALT mmol(g/l), aspartateaminotransferaze AFT mmol/(g/l) on biochemical analyzer using reactives on "Pliva-Lakhema" (Czech Republic). Glycerol-induced level in rats was developed by intramuscular injection of 50 % of glycerol solution (based on sodium chloride) in 10 ml/kg, 30–40 minutes after the last intake of drugs [2, 9]. 24 hours after toxic agent intake, excretory function of kidneys was investigated during water load (gastric intake of water of RT in 3 % of the total body weight along with urine test during 2 hours). After that, animals were sacrificed by decapitation under the narcosis. For biochemical studies, blood was collected and plasma was received, using heparin as anticoagulant. Creatinine was detected in blood plasma and urine (Jaffe reaction), urea was detected (diacetylmonoxime reaction). Proteins' concentration in urine was detected by sulphosalicylic acid [2]. Glomelural filtration, water reabsorption, creatinine excretion, urea, protein, clearance urea was calculated. Photometric measurements were done by spectrophotometer LabAnalyt SP-V1000 (Almedica, Ukraine).

Experimental data were statistically processed by STATISTICA 8.0 with calculation of mean values, by calculation of SEM (standard error of mean), significant point (p). Accuracy between-group differences depending on distribution was evaluated by Student's t-test, or by Mann-Whitney U-test, alternative indices by Fischer method. Changes were statistically significant at $p \leq 0.05$ [4].

Results of the study and their discussion. For studying hepatoprotective properties in substance 18, its action of the acute tetrachloronethane hepatitis was assessed. On the 4th day the development of experimental pathology was accompanied with the inhibition of the rats'general activity, decrease of water and food intake, changes of outer furs of animals and (eradication) death of 50 % of experimental animals (table 1). Formation of tetrachloronethane hepatitis caused enlargement of the liver mass coefficient (MC) by 1.4 ($p<0.01$) in comparison with the similar index in intact groups.

Table 1

Investigation of hepatoprotective properties of substance 18 and "Armadyne" during acute tetrachloronethane hepatitis (M±m, n=5–10).

Groups of animals	Coefficient of liver mass, %	Time of thiopental narcosis, min	Alanine-aminotransferase ALT mmol (g/l)	Aspartateaminotransferaze AFT mmol/(g/l)
Intact control	2.98±0.05	3.58±0.14	0.31±0.02	0.79±0.04
Tetrachloronethane hepatitis (control pathology)	4.08±0.20*	1.79±0.37*	1.64± 0.06*	2.91±0.18*
Tetrachloronethane hepatitis + "Armydene", 100 mg/kg	3.67± 0.10*	2.82± 0.17*,**	1.34± 0.04*,**	2.09± 0.19*,**
Tetrachloronethane hepatitis+substance 18, 12 mg/kg	3.43± 0.13*,**	1.31± 0.14*,**,#	1.06± 0.05*,**,#	1.70± 0.08*,**

Notes: 1. * – $p<0.05$ compared with intact control; 2. ** – $p<0.05$ compared with tetrachloronethane hepatitis; 3. # – $p<0.05$ compared with group glycerol+"Armadyne"; 4. n – number of animals in group.

At the same time the onset of thiopental anesthesia decreased by 2.0 times ($p<0.001$) compared to the value of the indicator in intact rats (table 1). In case of tetrachloromethane hepatitis, the activity of marker enzymes of hepatocyte cytolysis increased in the blood serum. At the end of the study ALT activity increased by 5.3 times ($p<0.001$), and AST activity – by 3.7 times ($p<0.001$) compared to the values in the intact control group.

Prophylactic administration of the reference drug "Armadyne" at the dose of 100 mg/kg improved the appearance, motor activity, food and fluid intake and partially reduced the mortality rate of rats by 30 % compared to that of experimental pathology. There was also a tendency to decrease the relative weight of the liver. The administration of ethylmethylhydroxypyridine succinate at the dose of 100 mg/kg contributed to the prolongation of the onset time of thiopental anesthesia by 1.8 times compared to that in the group of

rats with control pathology ($p<0.05$). In addition, administration of the "Armadyne" reference drug apparently reduced ALT activity in the blood serum by 1.6 times ($p<0.001$) and AST activity – by 1.5 times ($p<0.001$) compared to the values in the control pathology group.

Preventive use of compound 18 (12 mg/kg) against the background of acute terachloromethane hepatitis had a positive effect on the course of the experimental pathology. The appearance of rats, their general motor activity, behavior, and amount of food intake did not differ significantly from that in the intact control group. The administration of the compound contributed to the 100 % survival of the animals. The value of the liver relative weight decreased by 1.2 times ($p<0.02$) compared to the values in the control pathology group (table 1). The use of compound 18 apparently accelerated the onset of thiopental anesthesia in rats, which was obviously associated with the potentiating effect of the substance. Also, the substance probably reduced the development of hyperenzymemia. This was evidenced by a decrease in the activity of ALT and AST in blood serum by 1.6 times ($p<0.001$) and by 1.7 times ($p<0.001$) compared to the indices of rats with tetrachloromethane hepatitis. It should be noted that compound 18 was more effective than "Armadyne" in reducing ALT activity in blood serum by 1.3 times ($p<0.001$).

The results obtained indicate that compound 18 exhibits hepatoprotective properties which can be caused by an antioxidant activity and by a positive effect on metabolic processes in the liver, as it is shown under conditions of other pathology.

To establish the nephroprotective effect of the experimental compound, a model of glycerol-induced acute kidney injury in rats was used. It was found that, in 24 hours after the reproduction of acute renal failure, no deaths of rats were recorded in either the control pathology group or in the groups of animals receiving compound 18 and the reference drug. Experimental pathology was characterized by typical changes in excretory function of the kidneys and biochemical markers in blood plasma. The decrease in urination observed in animals during the introduction of water loading was associated with a drop in GFR (by 6.4 times) and water reabsorption ($p<0.05$ relative to intact control; table. 2).

Table 2

Effect of compound 18 and "Armadyne" on the excretory function of rat kidneys with glycerol-induced acute kidney injury under water loading conditions (M±m, n=6).

Experimental conditions, drugs	Intact control,	Control pathology (Glycerol)	Glycerol+"Armadyne", 100 mg/kg	Glycerol+compound 18, 12 mg/kg
Diuresis, ml/100 g for 2 hours	2.62±0.21	1.28±0.31*	1.20±0.25*	0.71±0.17*
Unloading, %	87.4±7.0	42.5±10.2*	39.9±8.5*	23.6±5.8*
GFR, ml/min per 100 g	0.159±0.057	0.025±0.006*	0.040±0.012*	0.020±0.006*
Water reabsorption, %	98.1±0.5	96.0±0.8*	97.0±1.0	96.5±0.8
Protein concentration in urine, g/l	0.12±0.04	1.02±0.27*	1.24±0.40*	0.75±0.15*
Protein excretion, mg/100 g for 2 hours	0.28±0.07	1.08±0.30*	1.15±0.22*	0,44±0.09#
Creatinine excretion, mcM/100 g for 2 hours	5.23±0.67	2.52±0.76	4.06±0.6	1.90±0.32*,#
Urea excretion, mcM/100 g for 2 hours	178.9±17.8	148.3±47.6	142.0±17.7	88.2±18.0*
Urea clearance, ml/min per 100 g	0.27±0.08	0.10±0.04	0.12±0.03	0.09±0.03*

Notes: 1. * – $p<0.05$ compared to the intact control; 2. # – $p<0.05$ compared to the glycerol+"Armadyne" group; 3. n – number of animals in the group.

Impairment of excretory function of the kidneys and mechanisms for overcoming hyperazotemia were manifested by an increase in the concentration of creatinine and urea in blood plasma compared to intact control by 2.7 and 2.6 times, respectively ($p<0.05$; table 2). Another unfavorable prognostic factor was the development of proteinuria: the concentration of protein in the urine apparently increased by 8.5 times and its excretion – by 3.9 times. Creatinine and urea excretion as well as urea clearance were not statistically different from those in intact animals although there was a noticeable decreasing trend of these specific indices.

Rats received the reference drug "Armadyne" were virtually indistinguishable from the animals of the control pathology group in almost all health outcome measures of excretory function of the kidneys. The content of creatinine and urea in blood plasma also approached the values registered in untreated animals.

Under the conditions of glycerol-induced acute kidney injury, the administration of compound 18 to rats resulted in more pronounced reduction in urination than in animals of the control pathology group and the "Armadyne" group. Obviously, this was associated with a decrease in GFR. However, the differences between the groups were not statistically significant. Hyperazotemia was observed, the retentive nature of which is evidenced by an accurate decrease in the excretion of creatinine and urea as well as a decrease in the renal clearance of the latter compared to the indices of intact animals.

Unlike the reference drug, compound 18 had an antiproteinuric effect; the excretion of protein in the urine was not significantly different from that in intact animals. Its concentration tended to decrease, although it remained high.

Received results have indicated that substance 18 detects hepatoprotective properties, which can be accompanied with antioxidant activity and positive influence on metabolic processes in the liver as well as defined in other pathology [11]. Also in derivatives of 2-oxyindoline-3-glyoxylic acid it has been indicated an ability to perform the role of antagonist and modulators of cAMP and set into the action the cascade of biochemical transformations of different enzymatic systems [5]. In substances of this group, significant antihypoxic action was determined [8]. One of the derivative of 2-oxoindoline due to stabilization of biological membranes was correlated with ethylmethyldroxypyridine succinate. This substance detected organoprotective action during cardiac pathology [1, 5, 8], which is conditioned by the correction of energy formation, the correlation between components of adenylic acid, and enzymatic stages of glycolysis and stages of carbohydrate metabolism [8, 9]. Obviously, the hepatoprotective action of compound 18 may be influenced by the above-mentioned metabolic units, which are primarily damaged by tetrachloromethane hepatitis.

The results obtained have indicated that, in addition to the positive effects on the liver, substance 18 did not detect protective effect under conditions of acute glycerol-induced kidney damage and, even, some indices were damaged. This may be due to the peculiarities of metabolic processes in the internal organs and indicate the possible side effects when using this substance, especially at high doses. It should be noted that among the 2-oxyindoline derivatives were found substances with diuretic action.

Conclusions

1. Under conditions of acute tetrachloromethane hepatitis, compound 18 (12 mg/kg) exhibits hepatoprotective activity that is indicated by a decrease in the liver mass coefficient and marker enzymes of hepatocyte injury in the blood serum.

2. The administration of compound 18 did not correct glycerol-induced acute kidney injury in rats, which is accompanied by impairment of excretory function of kidneys and proteinuria.

Prospects of further research: *in further studies, it is planned to study the effectiveness of 2-oxyindoline-3-glyoxylic acid derivative in hepatic encephalopathy, as well as to establish possible mechanisms of hepatoprotective action of 2-oxyindoline-3-glyoxylic acid derivative.*

References

1. Drachuk V.M. Nefroprotektorna aktyvnist pokhidnykh sirkovmisnykh aminokyslot (ademetioninu, taurynu ta hlutationu) za umov eksperymentalnoho hostroho poshkodzhennia nyrok [dysertatsia]. Kharkiv: Natsionalnyi Farmatsevtychnyi Universytet; 2019. 25s. [in Ukrainian]

2. Kamyshnykov V, Redaktor. Metody klynycheskykh laboratornykh yssledovanyi. Yzd. 10-E. Moskva: MEDpress-Ynform; 2020. 736s. [In Russian]

3. Kovalenko V, Viktorova A, redaktory. Kompendyum 2018 – lekarstvennye preparaty. K.: MORYON; 2019. 2560s. [in Russian]

4. Leonov V, redaktor. Nagliadnaya meditsinskaya statistika ucheb. Posobiye per. S anhl. Izd. 3-E Pererab. y dop. Moskva HEOTAR-Medya; 2015. 216s. [In Russian]

5. Markina A, Mishchenko O. Skriningovye issledovaniya novykh proizvodnykh 2-oksoyndolyna. Farmakolohiya ta likarska toksykolohiya. 2017;3(54):69–73. [in Russian]

6. Mironov A, redaktor. Rukovodstvo po provedenyiu doklinicheskikh issledovaniy lekarstvennykh sredstv. Moskva: Hryf y K; 2012. 944s. [In Russian]

7. Razumnyi R, Spirina I. Osoblyvosti psykhichnoho statusu khvorykh na nehospitalnu pnevmoniyu, spoluchenu z khronichnoyu patolohiieyu hepatobiliarnoyi systemy nevirusnoho henezu. Medychni perspektyvy. 2017;1:68–75. [in Ukrainian]

8. Redkin R, Chernykh V, Shemchuk L, Tsubanova N, Shtryhol C. Doslidzhennia zalezhnosti “khimichna struktura – antyhipoksychna diya” v riadu pokhidnykh indolu ta 2-oksindolu yaki mistiat etylaminovyi frahment. Zhurnal orhanichnoyi ta farmatsevtychnoyi khimiyi. 2014;12(1):28–38. [in Ukrainian]

9. Tovchyha O, Shtryhol S, Larianovska Yu. Vplyv nastoiky yahlytsi zvychainoyi (Aegopodium podagraria l.) ta metforminu na histostrukturu ta funktsiyu nyrok shchuriv z aloksanovym diabetom. Farmakolohiya ta likarska toksykolohiya. 2018;1:45–58. [in Ukrainian]

10. Chaikovska M, Martynyuk L. System inflammation and protein energy wasting correction in patients with chronic kidney disease. Ukrainian Journal of Nephrology and Dialysis. 2019; 2(62):41–47.

11. Lutsenko R, Vakhnenko A, Vlasova E. Research of the protection actions of derived 2-oxoindole in acute stress. Wiadomosci Lekarskie. 2017;30(1):57–61.

12. Lutsenko R, Vlasova E, Kolot E, Gladka V, Sidorenko A. The exchange of monoamines during the experimental neurosis on the background of using of amide «2-hydroxy-n-naphthalen-1-yl-2-(2-oxo-1,2-dihydroindol-3-ylidene)».Wiadomosci Lekarskie. 2017;30(5): 895–900.

13. Monamy V. Animal experimentation: a guide to the issues. 3 edition. New York: Cambridge; 2017. 125 p.

14. Shirazian S, Grant C, Aina O, Mattana J, Khorassani F. Ricardo A. Depression in chronic kidney disease and end-stage renal disease: similarities and differences in diagnosis, epidemiology, and management. Kidney Int Rep. 2017;2:94–107.

15. Söderlund J, Lindskog M. Relevance of rodent models of depression in clinical practice: can we overcome the obstacles in translational neuropsychiatry? International Journal of Neuropsychopharmacology. 2018;7:668–676.

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