https://doi.org/10.15360/1813-9779-2023-5-2337

Overtime Histological Changes in the Lungs after Intoxication with Baclofen Alone or in Combination with Ethanol (Experimental Study)

Olga L. Romanova[1,2]*, Mikhail L. Blagonravov[1], Pavel G. Dzhuvalyakov[1,2], Vladimir I. Torshin[1], Anton V. Ershov[3], Evgeniy Kh. Barinov[1]

[1] Patrice Lumumba Peoples Friendship University of Russia,
6 Miklukho-Maclaya Str., 117198 Moscow, Russia
[2] Avtsyn Research Institute of Human Morphology, Petrovsky Russian Scientific Center for Surgery
3 Tsyurupa Str., 107031 Moscow, Russia
[3] V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia

For citation: *Olga L. Romanova, Mikhail L. Blagonravov, Pavel G. Dzhuvalyakov, Vladimir I. Torshin, Anton V. Ershov, Evgeniy Kh. Barinov*. Overtime Histological Changes in the Lungs after Intoxication with Baclofen Alone or in Combination with Ethanol. *Obshchaya Reanimatologiya = General Reanimatology*. 2023; 19 (5): 53–58. https://doi.org/10.15360/1813-9779-2023-5-2337 [In Russ. and Engl.]

***Correspondence to:** Olga L. Romanova, olgpharm@yandex.ru

Summary

The aim of the study was to evaluate the overtime histological changes in the lungs after intoxication with baclofen alone or in combination with ethanol.

Materials and methods. The experiment was carried out on 35 male Wistar rats weighing 290–350 g and aged 20 weeks. The animals were split into 7 equal groups (n=5); test drugs were administered via nasogastric tube: rats from Groups 1, 3 and 5 were treated with baclofen at 85 mg/kg; rats from Groups 2, 4 and 6 received similar dose of baclofen and 40% alcohol by volume at a dose of 7 ml/kg; control group rats were not administered with any drugs. Animals of all groups were removed from the experiment by cervical dislocation under anesthesia (chlorolase) after 3 h (Groups 1, 2), 4.5 h (Groups 3, 4) and after 24 h (Groups 5, 6, and the controls). Lung tissue samples were examined by light microscopy. The nonparametric Kraskel–Wallis test was used for multiple comparisons between the groups, and nonparametric Mann–Whitney test with Bonferroni correction was used for pairwise comparison.

Results. Light microscopy showed no pathological changes in the lungs of the Control group animals. Baclofen alone, or in combination with ethanol caused significant circulatory disorders (venular and capillary fullness, hemorrhages in the interalveolar septa (IAS) and alveoli, sludge phenomenon), emphysema, atelectasis and distelectasis, and pulmonary edema. IAS thickness in rats from all experimental groups was different from that in animals from the Control group, all differences confirmed by the Kruskel–Wallis test: H=748, P=0.00001.

In Group 1 animals IAS was 44.2% thinner (P=0.00052) vs the control Group, while in all remaining experimental groups it was, on the contrary, thicker: in Group 2 — 57.6% increase in thickness (P=0.000038), in Group 3 — 99 % (P=0.00001), in Group 4 — 2.2-fold increase (P=0.00001), in Group 5 — 2.1-fold (P=0.00001), in Group 6 — 2.5-fold increase (P=0.00001). Most significant increase in IAS thickness (6-fold, P=0.00001) occurred within the period from 3 to 4.5 h after administration of baclofen, while within the period from 4.5 to 24 h no statistically significant increase occurred (P=0.99). Co-administration of baclofen and ethanol caused 2.8-fold (P=0.00001) increase in IAS thickness after 3 h as compared to the effects of baclofen only. IAS thickness at 4.5 h after baclofen and ethanol co-administration increased by additional 41.8% as compared to thickness at 3 h (P=0.00001). IAS became 11.8% thicker at 24 h vs 4.5 h (P=0.87). At 24 h IAS was 21.7% (P=0.0011) thicker after baclofen and ethanol co-administration vs baclofen alone. The alveoli size increased by 69.4% (P=0.00001) in Group 1 animals vs the Control group, by 14.3% (P=0.43) — in Group 2, by 55% (P=0.00004) — in Group 3, by 26.3% (P=0.002) — in Group 4, by 45% (P=0.0003) — in Group 5 (baclofen, 24 h), by 43.3% (P=0.0004) — in Group 6 (baclofen and ethanol, 24 h). Co-administration of baclofen and ethanol initially caused a slight increase in alveoli size, bur 3 h later there was a visible shrinkage in the diameter of alveoli by 32.5% (P=0.003) vs baclofen mono, 4.5 h later — by 18.5% (P=0.062), and 24 h later — by 1.2% (P=0.99), that is, the differences were leveled.

Conclusion. The combined effects of baclofen and ethanol induce more severe alterations in pulmonary tissue compared to baclofen alone. The pathological changes in the lungs reached their maximum by 24 h, which confirmed by morphometric assessment. Morphological changes in pulmonary tissue alongside with established chemical properties of the two agents can be used to diagnose cases of intoxication either with baclofen alone or in combination with ethanol.

Keywords: baclofen; ethanol; lungs; histological changes; morphological changes; intoxication

Conflict of interest. The authors declare no conflict of interest.

Introduction

Poisoning is one of the leading causes of violent death nowadays [1–3]. The muscle relaxant baclofen is a common cause of poisoning [4–6]. Baclofen, unlike other substances in this class, is a β-p-chlorophenyl derivative of GABA (gamma-aminobutyric acid) [7–9]. Baclofen is a prescription medication available in both oral and intrathecal forms [7, 8].

Oral baclofen is indicated for the treatment of severe muscle spasticity, multiple sclerosis, tumors, trauma, spinal cord infections, acute cerebrovascular accidents, and meningitis. The efficacy of baclofen in patients with alcoholism [10–14] and drug addiction [14] has been studied, and several studies have shown its benefit in cerebral palsy [15, 16].

Baclofen has significant psychoactive effects [17–20]. For this reason, it is widely used by drug addicts, especially young people [21]. To achieve a narcotic effect, baclofen doses are increased many times over, up to 6–14 tablets. The drug is often combined with low-alcohol drinks. In this case, narcotic intoxication occurs in about half an hour. The main symptoms are nausea and vomiting, dizziness, impaired motor coordination, drowsiness, slurred speech [17–20].

Significant overdose with baclofen can lead to acute toxicity and death [18, 20, 22]. There is no specific antidote for poisoning with this drug [23].

In all suspected cases of baclofen poisoning, differential diagnosis with other poisonings is necessary for the most effective rehabilitation. A comprehensive understanding of the pathophysiology of the different stages of baclofen poisoning could help to provide timely help to this category of patients. In the case of fatal baclofen poisoning, a toxicologic investigation is necessary to determine the immediate cause of death [18, 22].

According to the literature, the lung is one of the target organs in baclofen poisoning [24]. The combined effect of baclofen and ethanol on the lung has been poorly studied.

The aim of the study was to evaluate the histologic changes in the lungs during baclofen or baclofen-ethanol poisoning.

Materials and methods

Thirty-five adult (20 weeks old) male Wistar rats weighing 290–350 g were included in the experiment. The animals were divided into 7 groups (5 rats in each group).

The experiments were conducted in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes [25].

Baclofen or its combination with ethanol was administered to animals under general anesthesia (chloralose) via a gastric tube.

Animals were divided into the following groups:

- Control (n=5) —animals receiving neither baclofen nor ethanol;
- Group 1 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 3 h;
- Group 2 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 3 h;
- Group 3 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 4.5 h;
- Group 4 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 4.5 h;
- Group 5 (n=5) — animals receiving baclofen 85 mg/kg, duration of experiment 24 h;
- Group 6 (n=5) — animals receiving a combination of baclofen 85 mg/kg and 40% ethanol 7 ml/kg, duration of experiment 24 h.

After drug administration, animals were awakened from anesthesia and left in the animal facility with free access to water but without food. After 3, 4, 5 and 24 h, animals were euthanized by cervical dislocation under anesthesia (chloralose). The thoracic cavity was opened and the lungs were removed and placed in 10% neutral formalin and embedded in paraffin. Lung sections, 5 μm thick, were mounted on slides and stained with hematoxylin and eosin using standard techniques. Histologic preparations were examined at ×400 magnification. A Nikon E-400 microscope with a video system based on a Watec 221S camera was used. Signs of impaired circulation (arterial, venous and capillary hemorrhage, sludge phenomenon, interalveolar septal hemorrhage, alveoli), atelectasis, dystelectasis and emphysema, fluid in bronchiolar lumen, epithelial desquamation in bronchiolar lumen, thickening of interalveolar septa due to edema were evaluated. Fisher's criterion was used to evaluate the statistical significance of histologic signs. A histologic feature was considered significant if it was observed in 4 or 5 cases in one group and in none in the other. Further morphometric examination of the specimens was performed using ImageScope 12.0. Alveolar diameter and interalveolar septa thickness were measured. We performed 30 measurements in each animal, so the sample contained 150 measurements in each group. The Shapiro–Wilk test showed a non-normal distribution of the obtained data, so the data were presented as median, lower and upper quartiles *[Me (QL;QH)]*. The nonparametric Kruskal–Wallis test was used for multiple comparisons between groups, and the nonparametric Mann–Whitney test with Bonferroni correction was used for pairwise comparisons. The number of pairs of comparisons was 13, and the critical significance level was 0.0038. Microsoft Excel and Statistica 12.0 software were used for statistical analysis of the data [26, 27].

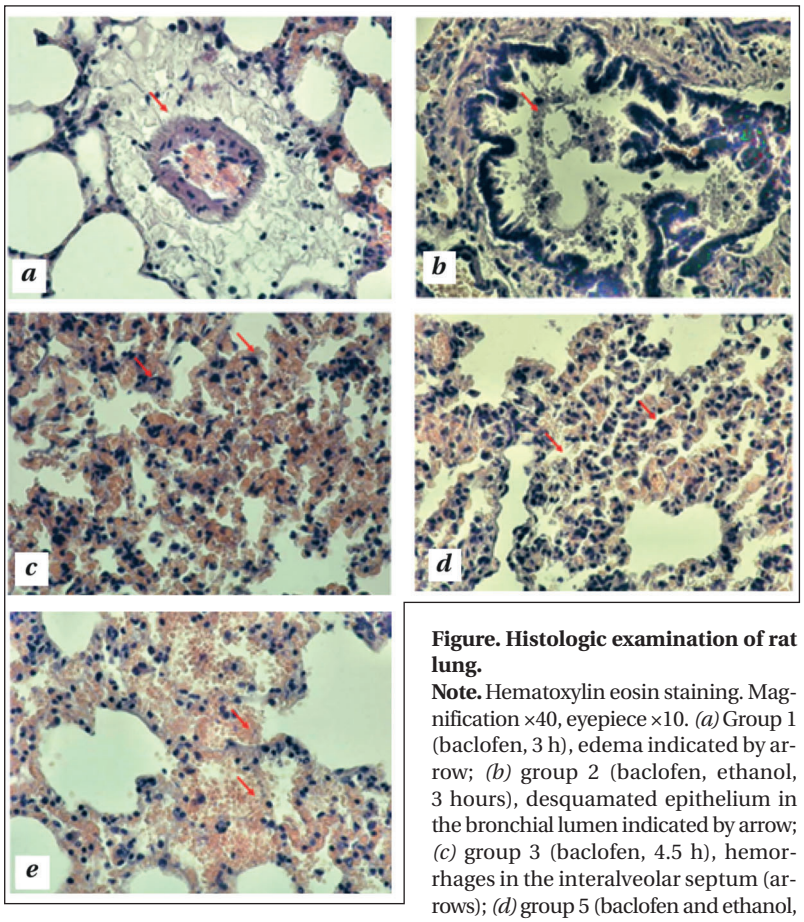

Figure. Histologic examination of rat lung.
Note. Hematoxylin eosin staining. Magnification ×40, eyepiece ×10. *(a)* Group 1 (baclofen, 3 h), edema indicated by arrow; *(b)* group 2 (baclofen, ethanol, 3 hours), desquamated epithelium in the bronchial lumen indicated by arrow; *(c)* group 3 (baclofen, 4.5 h), hemorrhages in the interalveolar septum (arrows); *(d)* group 5 (baclofen and ethanol, 4.5 hours), dystelectasis (arrows); *(e)* group 6 (baclofen, ethanol, 24 h), alveolar hemorrhages (arrows).

Results

No pathologic changes were observed in the lungs of rats of the control group.

The data obtained during the study of lungs of animals of group 1 confirm the results of our previous experiments [6]. Blood circulation disorders (venous and capillary hemorrhage), emphysema, atelectasis and dystelectasis, cellular response, thickening of interalveolar septa due to edema were noted in the lungs of animals of group 1 (Figure, *a*).

The data obtained when examining the lungs of group 2 animals also confirm the results of our previous experiments [6]. In addition to the histological changes described above for group 1, the presence of secretion and epithelial desquamation in the lumen of bronchioles was observed in the bronchi of animals in this group (Figure, *b*).

In group 3, characteristic features included arterial, venous and capillary congestion, sludge and hemorrhage in the IAS, which was not observed in the control group, neither in group 1 nor in group 2 (Figure, *c*). In addition, atelectasis, dystelectasis, IAS thickening due to edema, and emphysematous areas with thin IAS were observed in this group.

Blood circulation disturbances (venous, capillary, arterial hemorrhages, sludge, hemorrhages in IAS) were observed 4.5 h after administration of the combination of baclofen and ethanol. We also observed atelectasis and dystelectasis (Figure, *d*), emphysema (in the areas of thin IAS), fluid and desquamation of epithelium in bronchioles. All of these histologic changes were significant. Isolated hemorrhages appeared in the alveoli, which was not observed in group 1 or group 2.

In group 5 we observed venous and arterial congestion, sludge, hemorrhage in IAS. Atelectasis and dystelectasis, IAS thickening due to edema and emphysema were found. All of the above signs were significant. Isolated alveolar hemorrhage was also observed.

In group 6, the characteristic histological feature included venous, capillary and arterial hemorrhage, hemorrhage in IAS and in alveoli (Figure, *e*). Emphysema developed in the lungs of animals in this group. In addition, thickening of the IAS due to edema was observed. Fluid in the bronchioles and desquamation of the epithelium into the bronchial lumen were observed. Fluid in the bronchial lumen and epithelial desquamation in the bronchial lumen were observed only in the groups receiving both baclofen and ethanol (groups 2, 4, 6), but not in the groups receiving baclofen alone (groups 1, 3, 5).

The results of the morphometric study of the lungs after administration of baclofen and its combination with ethanol are shown in the Table.

Table. Interalveolar septum (IAS) thickness and alveolar diameter after administration of baclofen and its combination with ethanol, *Me* (*LQ; HQ*).

Group	Values	
	Thickness of IAS, μm	Alveolar diameter, μm
Control	7.7 (6.2; 9.3)	41.5 (35.2; 51.6)
1	4.3 (3.8; 5.1)[c]	70.2 (54.0; 86.3)[c]
2	12.2 (10.5; 13.9)[c,1]	47.4 (37.6; 56.3)[1]
3	15.4 (13.6; 17.9)[c,2]	64.3 (55.6; 75.0)[c,2]
4	17.3 (14.9; 19.9)[c,2,3]	52.4 (45.2; 60.2)[c]
5	15.9 (13.9; 18.4)[c,2,3]	60.1 (52.1; 70.6)[c]
6	19.4 (15.3; 22.8)[c,2,3,4]	59.4 (50.1; 69.3)[c]

Note. Differences are significant versus: [c] — control; [1] — group 1; [2] — group 2; [3] — group 3; [4] — group 4 at $P<0.0038$ (Mann–Whitney test).

The table shows that the IAS thickness in all experimental groups was different from the control. The Kruskal–Wallis test confirmed the existence of differences with H=748, P=0.00001. Meanwhile, IAS thickness in group 1 was 44.2% (P=0.00052) lower than in the control group, whereas in the other

groups it was higher: in group 2 by 57.6% (P=0.000038), in group 3 by 99% (P=0.00001), in group 4 by 2.2 times (P=0.00001), in group 5 by 2.1 times (P=0.00001), in group 6 by 2.5 times (P=0.00001). From 3 to 4.5 h after baclofen administration, a 3.6-fold increase in IAS thickness was observed (P=0.00001). No significant differences in IAS thickness were observed at 4.5 and 24 h (P=0.99). At 3 h after co-administration of baclofen and ethanol, a 2.8-fold (P=0.00001) increase in IAS thickness was observed compared to baclofen alone. IAS thickness 4.5 h after baclofen and ethanol administration was 41.8% higher than at 3 h (P=0.00001), and 24 h later it was 11.8% higher than at 4.5 h (P=0.87). Twenty-four h after administration of baclofen with ethanol, IAS thickness was 21.7% greater (P=0.0011) than after administration of baclofen alone.

Alveolar diameter was 69.4% greater (P=0.00001) in group 1, 14.3% greater (P=0.43) in group 2, 55% greater (P=0.00004) in group 3, 26.3% greater (P=0.002) in group 4, 45% greater (P=0.0003) in group 5 (baclofen, 24 h), and 43.3% greater (P=0.0004) in group 6 (baclofen and ethanol, 24 h) compared to the control group. A slight increase in alveolar diameter was observed after co-administration of baclofen and ethanol. At 3 h after co-administration of baclofen and ethanol, the alveolar diameter was 32.5% (P=0.003) smaller than after administration of baclofen alone, at 4.5 h by 18.5% (P=0.062), at 24 h by 1.2% (P=0.99), i.e., the differences disappeared.

Discussion

Baclofen administration decreases the tone of skeletal muscles, including the intercostal muscles. Excessive relaxation of these muscles leads to respiratory compromise and subsequent hypoxia [4, 7]. Baclofen is known to be a selective inhibitor of $GABA_B$ receptors, but at high enough doses it can cause stimulation of $GABA_A$ receptors, resulting in contraction of smooth muscle of bronchi and bronchioles with subsequent spasm and respiratory distress. In addition, stimulation of these receptors has been shown to increase vascular and tissue permeability [28]. In animal studies, we found that rats receiving baclofen alone (groups 3, 5) and a combination of baclofen and ethanol (groups 2, 4, 6) had significantly greater IAS thickness than the control group (except group 1), possibly due to stimulation of $GABA_A$ receptors by a subtoxic dose of baclofen and the development of hypoxia [29–30].

Thus, in all groups receiving a combination of baclofen and ethanol (2, 4, 6), IAS thickness was significantly greater than in the groups receiving baclofen alone. This confirms the hypothesis of a combined negative effect of baclofen and ethanol on the architecture of the air-blood barrier. As a result, the likelihood of impaired oxygen diffusion and more severe hypoxia increases.

Conclusion

After administration of the myorelaxant baclofen alone or in combination with ethanol, circulatory disturbances (venous and capillary hemorrhage, IAS and alveolar hemorrhage, sludge), emphysema, atelectasis and dystelectasis develop. With increasing hypoxia, vascular and tissue permeability increases and edema develops. The combined effect of baclofen and ethanol causes more severe changes in the lungs (epithelial desquamation and fluid in the bronchial lumen were observed only in the groups of animals receiving a combination of baclofen and ethanol). The pathological changes in the lungs showed progression, reaching the maximum severity at 24 h, which was confirmed by the results of the morphometric study. Data on morphological changes in the lungs can be extrapolated to the forensic material and later, together with the results of chemical tests, can be used to diagnose intoxication by baclofen and its combination with ethanol, as well as to determine the mode of drug administration (alone or in combination with ethanol).

References

1. *Симонова А.Ю., Ильяшенко К.К., Клычникова Е.В., Евсеев А.К., Поцхверия М.М., Белова М.В., Тазина Е.В., Шабанов А.К., Кузовлев А.Н.* Оценка оксидантно-антиоксидантной системы крови у гериатрических пациентов с острыми отравлениями. *Общая реаниматология.* 2022; 18 (3): 38–44. [*Simonova A.Yu., Ilyashenko K.K., Klychnikova E.V., Evseev A.K., Potshveria M.M., Belova M.V., Tazina E.V., Shabanov A.K., Kuzovlev A.N.* Parameters of the blood oxidant/antioxidant system in elderly patients with acute poisoning. *General Reanimatology/Obshchaya Reanimatologya.* 2022; 18 (3): 38–44. DOI: 10.15360/1813-9779-2022-3-38-44. (in Russ.)]. DOI: 10.15360/1813-9779-2022-3-38-44.
2. *Синенченко А.Г., Батоцыренов Ч.Б., Лодягин А.Н., Синенченко Г.И., Коваленко А.Л.* Делирий при острых отравлениях 1,4-бутандиолом и его коррекция. *Общая реаниматология.* 2021; 17 (6): 42–48. [*Sinenchenko A.G., Batotsyrenov C.B., Lodyagin A.N., Sinenchenko G.I., Kovalenko A.L.* Delirium in acute poisoning with 1,4-butanediol and its correction. *General Reanimatology/Obshchaya Reanimatologya.* 2021; 17 (6): 42–48. (in Russ.)]. DOI: 10.15360/1813-9779-2021-6-42-48.
3. *Romanova O.L., Chauhan M., Blagonravov M.L., Kislov M.A.· Ershov. A.V., Krupin K.N.* Baclofen (fun drug) and ethanol combined poisoning in humans: A histopathology and morphometry model. *J Forensic Leg Med.* 2022; 90: 102373. DOI: 10.1016/j.jflm.2022.102373. PMID: 35671675.
4. *Moffat A.C., Osselton M.D., Widdop B. (eds.).* Clarke's analysis of drugs and poisons. London: Pharmaceutical Press; 2011; edited 2020. ISBN: 0-853-69473-7.
5. *Романова О.Л., Сундуков Д.В., Голубев М.А., Благонравов М.Л., Гошкоев В.В., Чурилов А.А.* Патологические изменения в печени при острых отравлениях клозапином и его сочетанием с этанолом (экспериментальное исследование). *Общая реаниматология.* 2019; 15 (2): 27–35. [*Romanova O.L., Sundukov D.V., Golubev M.A., Blagonravov M.L., Goshkoev V.V., Churilov A.A.* Pathological changes in the liver during acute exposure to clozapine and its combination with ethanol (experimental study). *General Reanimatology/Obshchaya Reanimatologya.* 2019; 15 (2): 27–35. (in Russ.)]. DOI: 10.15360/1813-9779-2019-2-27-35.
6. *Романова О.Л., Сундуков Д.В., Голубев М.А., Благонравов М.Л., Ершов А.В.* Характеристика общепатологических процессов в легких при отравлении баклофеном. *Obshchaya Reanimatologiya = General Reanimatology.* 2021; 17 (2): 37–44. [*Romanova O.L., Sundukov D.V., Golubev M.A., Blagonravov M.L., Ershov A.V.* Lung histopathology in baclofen intoxication. *General Reanimatology/Obshchaya Reanimatologya.* 2021; 17 (2): 37–44. (in Russ.)]. DOI: 10.15360/1813-9779-2021-2-37-44.
7. Baclofen monograph for professionals. *Drugs.com.* American Society of Health-System Pharmacists. Retrieved 3 March 2019. https: //www.drugs.com/monograph/baclofen.html.
8. *Машковский М.Д.* Лекарственные средства. 17-е изд. М.: Новая волна; 2019: 73–74. [*Mashkovsky M.D.* Medicinal products. 17th ed. Moscow: New Wave/Novaya Volna; 2019: 73–74].
9. Gablofen (Baclofen). FDA full prescribing information. *US Food and Drug Administration.* Retrieved 2021. https: //fda.report/DailyMed/ 00d3e846-dd92-448d-9ab8-6a07be823cc1.
10. *Liu J., Wang L.N.* Baclofen for alcohol withdrawal. Cochrane Database of Systematic Reviews 2019; 11. Art. No.: CD008502. DOI: 10.1002/14651858.CD008502.pub6. Accessed 28 May 2023. https: //www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD008502.pub6/full#CD008502-abs-0006.
11. *Franchitto N., De Haro L., Pélissier F.* Focusing solely on the effect of the medication without taking a holistic view of the patient does not seem very constructive. *Clin Toxicol (Phila).* 2018; 56 (4): 309. DOI: 10.1080/15563650.2017.1373781. PMID: 28874088.
12. *Beck A., Pelz P., Lorenz R.C., Charlet K., Geisel O., Heinz A., Wüstenberg T., et al.* Effects of high-dose baclofen on cue reactivity in alcohol dependence: a randomized, placebo-controlled pharmaco-fMRI study. *Eur Neuropsychopharmacol.* 2018; 28 (11): 1206–1216. DOI: 10.1016/j.euroneuro.2018.08.507. PMID: 30217552.
13. *Girish K., Vikram Reddy K., Pandit L.V., Pundarikaksha H.P., Vijendra R., Vasundara K., Manjunatha R., et al.* A randomized, open-label, standard controlled, parallel group study of efficacy and safety of baclofen, and chlordiazepoxide in uncomplicated alcohol withdrawal syndrome. *Biomed J.* 2016; 39 (1): 72–80. DOI: 10.1016/j.bj.2015.09.002. PMID: 27105601.
14. *Bareli T., Ahdoot H.L., Ben Moshe H., Barnea R., Warhaftig G., Gispan I., Maayan R., et al.* Novel opipramol-baclofen combination alleviates depression and craving and facilitates recovery from substance use disorder-an animal model and a human study. *Front Behav Neurosci.* 2021; 15: 788708. DOI: 10.3389/fnbeh.2021.788708. PMID: 35002647.
15. *McLaughlin M.J., He Y., Brunstrom-Hernandez J., Thio L.L., Carleton B.C., Ross C.J.D., Gaedigk A., et al.* Pharmacogenomic variability of oral baclofen clearance and clinical response in children with cerebral palsy *PM R.* 2018; 10 (3): 235–243. DOI: 10.1016/j.pmrj.2017.08.441. PMID: 28867665.
16. *Lake W., Shah H.* Intrathecal baclofen infusion for the treatment of movement disorders. *Neurosurg Clin N Am.* 2019; 30 (2): 203–209. DOI: 10.1016/j.nec.2018.12.002. PMID: 30898271.
17. *Charifou Y., Martinet O., Jabot J., Gauzere B.A., Allyn J., Vandroux D.* Baclofen intoxication cases in an intensive care unit. *Anaesth Crit Care Pain Med.* 2016; 35 (2): 169–170. DOI: 10.1016/j.accpm.2015.10.003. PMID: 26667597.
18. *Дукова О.А., Покровский А.А., Мелентьев А.Б., Краснов Е.А., Суворова Е.В., Ефремов А. А.* Смертельное отравление баклофеном. *Судебно-медицинская экспертиза.* 2015; 58 (1): 35–39. [*Dukova O.A., Pokrovskij A.A., Melent'ev A.B., Krasnov E.A., Suvorova E.V., Efremov A.A.* Lethal intoxication with baclofen. *Forensic Medical Examination/Sudebno-Meditsinskaya Ekspertisa.* 2015; 58 (1): 35–39. (In Russ.)]. DOI: 10.17116/sudmed201558135-39.
19. *Pelerin J-M., Fristot L., Gibaja V., Revol B., Gillet P., Lima-Tournebize J.* Non-medical use of baclofen: a case series and review of the literature. *Therapie.* 2023; S0040-5957 (23)0035-5. DOI: 10.1016/j.therap.2023. 02.007. PMID: 36922285.
20. *de Marcellus C., le Bot S., Decleves X., Baud F., Renolleau S., Oualha M.* Report of severe accidental baclofen

intoxication in a healthy 4-year-old boy and review of the literature. *Arch Pediatr*. 2019; 26 (8): 475–478. DOI: 10.1016/j.arcped.2019.10.003.PMID: 31685412.
21. *Drevin G., Briet M., Ghamrawi S., Beloncle F., Abbara C.* Baclofen overdose following recreational use in adolescents and young adults: a case report and review of the literature. *Forensic Sci Int*. 2020; 316: 110541. DOI: 10.1016/j.forsciint.2020.110541. PMID: 33096455.
22. *Beraha E., Bodewits P., van den Brink W., Wiers R.* Speaking fluently with baclofen?. *BMJ Case Rep*. 2017: bcr-2016218714. DOI: 10.1136/bcr-2016-218714.PMID: 28495786.
23. *Ghannoum M., Berling I., Lavergne V., Roberts D.M., Galvao T., Hoffman R.S., Nolin T.D., et al.* EXTRIP workgroup. Recommendations from the EXTRIP workgroup on extracorporeal treatment for baclofen poisoning. *Kidney Int*. 2021; 100 (4): 720–736. DOI: 10.1016/j.kint.2021. 07.014. PMID: 34358487.
24. *Issa S.Y., Hafez E.M., El-Banna A.S., Abdel Rahman S.M., AlMazroua M.K., El-Hamd M.A.* Baclofen systemic toxicity: experimental histopathological and biochemical study. *Hum Exp Toxicol*. 2018; 37 (4): 431–441. DOI: 10.1177/0960327117712369. PMID: 28565970.
25. Directive 2010/63 / EU of the European Parliament and the Council of the European Union on the Protection of Animals used for scientific purposes. St. Petersburg: Rus-LASA NP «Association of Specialists Working with Laboratory Animals». https: //eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ: L: 2010: 276: 0033: 0079: en: PDF.
26. *Гланц С.А.* Медико-биологическая статистика. Пер. с англ. М.: Практика; 1998: 459. [*Glants S.A.* Primer of biostatistics. Trans. from English M.: Praktika; 1998: 459].
27. *Баврина А.П.* Современные правила использования методов описательной статистики в медико-биологических исследованиях. *Медицинский альманах*. 2020. 2 (63): 95–105. [*Bavrina A.P.* Modern rules for the use of descriptive statistics methods in biomedical research. *Medical Almanac/Meditcinsky Almanah*. 2020. 2 (63): 95–105 (in Russ.)]. eLIBRARY ID: 43118612. EDN: UCVXIX.
28. *Chapman R.W., Hey J.A., Rizzo C.A., Bolser D.C.* GABAB receptors in the lung. *Trends Pharmacol Sci*. 1993; 14 (1): 26–29. DOI: 10.1016/0165-6147 (93)90110-6. PMID: 8382886.
29. *Mizuta K., Xu D., Pan Y., Comas G., Sonett J.R., Zhang Y., Panettieri Jr. R.A., et al.* GABAA receptors are expressed and facilitate relaxation in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol*. 2008; 294 (6): L1206–16. DOI: 10.1152/ajplung.00287.2007. PMID: 18408071.
30. *Denora N, Laquintana V, Lopedota A, Serra M, Dazzi L, Biggio G, Pal D., et al.* Novel L-Dopa and dopamine prodrugs containing a 2-phenyl-imidazopyridine moiety. *Pharm Res*. 2007; 24 (7): 1309–1324. DOI: 10.1007/s11095-007-9255-y. PMID: 17404814.

Received 15.04.2023
Accepted 04.07.2023