UDC 615.234:616-002:616-001.18:616.853:615.015 https://doi.org/10.26641/2307-0404.2021.2.234486

S.Yu. Shtrygol,
I.G. Kapelka,
M.V. Mishchenko,
O.Ya. Mishchenko

NON-OBVIOUS EFFECTS OF MONTELUKAST – LEUKOTRIENE RECEPTOR BLOCKER: FRIGOPROTECTIVE AND ANTICONVULSANT PROPERTIES

National University of Pharmacy
Department of pharmacology and pharmacotherapy
Pushkinska str., 53, Kharkiv, 61002, Ukraine
Institute for Advanced Training of Pharmacy Specialists (IATPS) *
Department of clinical pharmacology
Zahysnykiv Ukrainy sq., 17, Kharkiv, 61001, Ukraine
Національний фармацевтичний університет
кафедра фармакології та фармакотерапії
(зав. – д. мед. н., проф. С.Ю. Штриголь)
вул. Пушкінська, 53, Харків, 61002, Україна
Інститут підвищення кваліфікації спеціалістів фармації *
Національного фармацевтичного університету
кафедра клінічної фармакології
(зав. – д. фарм. н., проф. О.Я. Міщенко)
майдан Захисників України, 17, Харків, 61001, Україна
e-mail: shtrygol@ukr.net

Цитування: Медичні перспективи. 2021. Т. 26, № 2. С. 19-25
Cited: Medicni perspektivi. 2021;26(2):19-25

Key words: *montelukast, inflammation, leukotrienes, cold trauma, epilepsy*
Ключові слова: *монтелукаст, запалення, лейкотрієни, холодова травма, епілепсія*
Ключевые слова: *монтелукаст, воспаление, лейкотриены, холодовая травма, эпилепсия*

Abstract. Non-obvious effects of montelukast – leukotriene receptor blocker: frigoprotective and anticonvulsant properties. Shtrygol S.Yu., Kapelka I.G., Mishchenko M.V., Mishchenko O.Ya. *The participation of arachidonic acid metabolism products – prostaglandins and leukotrienes – in the process of inflammation is a common pathogenetic link of cold injury and epilepsy. Montelukast is widely used for the treatment of bronchial asthma and allergic rhinitis as a leukotriene receptor blocker. However, the mechanism of action of the drug suggests a wider range of its pharmacological properties and the corresponding scope of application. This study is aimed to determine the effectiveness of montelukast as a potential frigoprotective and anticonvulsant drug. Experiments were performed on 73 white mice weighing 20-22 g on models of acute general cooling and pentylenetetrazol convulsions. Frigoprotective properties were studied at a temperature of -18°C, recording the lifetime. Montelukast ("Singular", 2 mg/kg), acetylsalicylic acid ("Aspirin", 50 mg/kg), celecoxib ("Celebrex", 74 mg/kg), diclofenac sodium ("Voltaren", 14 mg/kg) were administered intragastrically as a suspension in a prophylactic mode, 30 minutes before the cold injury. In the study of anticonvulsant activity, montelukast ("Singular", 4 mg/kg) and sodium valproate ("Depakin", 300 mg/kg) were administered intragastrically 30 minutes before stimulating convulsions by subcutaneous administration of pentylenetetrazole (90 mg/kg). The latent period of convulsions, the number of convulsions per 1 animal, % of mice with clonic and tonic paroxysms, the severity of convulsions in points, the duration of the convulsive period, the lifetime of animals and lethality were recorded for an hour. On the model of acute general cooling, montelukast showed a dose-dependent frigoprotective effect at a dose of 2 mg/kg surpassing drugs with proven frigoprotective properties – acetylsalicylic acid and celecoxib. On the model of pentylenetetrazole-induced convulsions, montelukast statistically significantly reduced the integral indicator of anticonvulsant activity – lethality – by 2.57 times. Thus, the experiment proved the significant role of leukotrienes in the pathogenesis of cold injury and epilepsy and justified the feasibility of further study of the frigoprotective and anticonvulsant properties of montelukast – leukotriene receptor blocker a drug as for adjuvant therapy, especially when these pathologies are combined with bronchial asthma and allergic rhinitis.*

Реферат. Неочевидные эффекты блокатора лейкотриеновых рецепторов монтелукаста: фригопротекторные и противосудорожные свойства. Штриголь С.Ю., Капелька И.Г., Мищенко М. В., Мищенко О.Я. *Участие продуктов метаболизма арахидоновой кислоты – простагландинов и лейкотриенов – в процессе*

воспаления является общим патогенетическим звеном холодовой травмы и эпилепсии. Монтелукаст как блокатор лейкотриеновых рецепторов широко применяется для лечения бронхиальной астмы и аллергических ринитов. Однако механизм действия препарата дает основания предположить более широкий спектр его фармакологических свойств и соответствующую область применения. Целью работы стало выяснение эффективности монтелукаста как потенциального фригопротекторного и противосудорожного препарата. Эксперименты проведены на 73 белых мышах массой 20-22 г на моделях острого общего охлаждения и пентилентетразоловых судорог. Фригопротекторные свойства изучали при температуре -18°С, регистрируя время жизни. Монтелукаст («Сингуляр», 2 мг/кг), ацетилсалициловую кислоту («Аспирин», 50 мг/кг), целекоксиб («Целебрекс», 74 мг/кг), диклофенак натрия («Вольтарен», 14 мг/кг) вводили внутрижелудочно в виде суспензии в профилактическом режиме за 30 мин. до холодовой травмы. При изучении противосудорожной активности монтелукаст («Сингуляр», 4 мг/кг) и вальпроат натрия («Депакин», 300 мг/кг) вводили внутрижелудочно за 30 мин. до моделирования судорог подкожным введением пентилентетразола (90 мг/кг). На протяжении часа регистрировали латентный период судорог, количество судорог на 1 животное, % мышей с клоническими и тоническими пароксизмами, тяжесть судорог в баллах, продолжительность судорожного периода, время жизни животных и летальность. На модели острого общего охлаждения монтелукаст проявил дозозависимый фригопротекторный ефект, превосходя в дозе 2 мг/кг препараты с доказанными фригопротекторными свойствами – ацетилсалициловую кислоту и целекоксиб. На модели пентилентетразоловых судорог монтелукаст статистически достоверно снижал в 2,57 раза интегральный показатель противосудорожной активности – летальность. Таким образом, в эксперименте доказана значительная роль лейкотриенов в патогенезе холодовой травмы и эпилепсии и обоснована целесообразность дальнейшего изучения фригопротекторных и антиконвульсантных свойств блокатора лейкотриеновых рецепторов монтелукаста как средства адъювантной терапии, особенно при сочетании указанных патологий с бронхиальной астмой и аллергическим ринитом.

The combination of various pathologies, such as cold trauma (CT) and epilepsy in the title of the article, may seem unexpected and even illogical. However, they are united by the presence of common features of pathogenesis, which may become a promising target of pharmacological effects, namely the activation of pro-inflammatory mechanisms. The relevance of CT is emphasized by the latest statistical and epidemiological data. In particular, in Ukraine only for the period from December 12, 2018 to January 8, 2019, 1037 people who suffered from hypothermia and frostbite applied to health care facilities, 955 people were hospitalized [17], and prevention and treatment of CT requires improvement. About 1% of the population suffers from epilepsy, which is about 70 million patients worldwide, a third of whom due to polypharmacoresistance can not achieve remission, despite the use of classic antiepileptic drugs [7, 10]. Therefore, in the pharmacotherapy of both CT and epilepsy, it is advisable to use additional drugs to expand the impact on key pathogenetic mechanisms, including pro-inflammatory.

Inflammation as a typical pathological process is a common pathogenetic link of many diseases, including CT and epilepsy. A special role in this process is played by the products of arachidonic acid metabolism - prostaglandins and leukotrienes.

In the case of CT, prostaglandins, namely prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2), increase platelet aggregation and vasoconstriction, leading to the formation of blood clots in the vascular bed and the development of ischemia [18]. Leukotrienes function as potent chemoattractants, increase vascular permeability, which accelerates the development of inflammation [18]. Fluid extravasation which naturally occurs under such conditions, is one of the main damaging factors of CT. Ice microcrystals formed in the intercellular space at low temperatures cause damage to old tissues and increase the area of the lesion [19]. However, information on the possible effectiveness of inhibitors of the leukotriene link of the arachidonic acid cascade in CT, in contrast to numerous data on the frigoprotective effect of inhibitors of the cyclooxygenase pathway, is missing.

The pathogenesis of epilepsy is closely linked to the development of neuroinflammation. Patients have increased levels of prostaglandins, leukotrienes B_4 and C_4 in the hippocampus and cerebrospinal fluid. Cysteinyl leukotriene D_4 (a $CysLT_1$ receptor agonist) promotes the development of pentylenetetrazole-induced seizures (PTZ), and the leukotriene receptor blocker montelukast with intracerebral administration reduces this effect. In addition, montelukast enhances the anticonvulsant effect of phenobarbital and inhibits leukocyte infiltration of the brain. There are isolated data on the anticonvulsant properties of montelukast in rats at doses of 25-100 mg/kg [15, 16], which, however, significantly exceed the average therapeutic doses per person [11].

Given the role of leukotrienes in the pathogenesis of CT and epilepsy and the inhibitory effect of

montelukast on the relevant inflammatory site, it is advisable to study the frigoprotective and anticonvulsant properties of this blocker of $CysLT_1$-leukotriene receptors.

The purpose of the study was to determine the effectiveness of montelukast as a means of frigoprotective and anticonvulsant action.

MATERIALS AND METHODS OF RESEARCH

Frigoprotective properties of montelukast (tablets "Singular", "MerckSharp & DohmeCorp", USA) were studied in a model of acute general cooling (AGC) on 36 white nonlinear mice of both sexes weighing 20-22 g. For modeling AGCct the mobility of animals and air access, they were placed into individual transparent plastic containers which did not restrict mobility and access for air, were put into a freezer "Nord Inter-300" with a transparent lid at -18° C. An integral indicator of frigoprotective action – lifetime was registered [6]. Acetylsalicylic acid (ASA, Aspirin tablets, Bayer, Germany), celecoxib (Celebrex tablets, Pfizer tablets, USA) and diclofenac sodium (Voltaren tablets, Novartis tablets, Switzerland) were selected as reference drugs, based on their frigoprotective activity data [6]. The tablets were crushed and suspended with the addition of tween-80, administered as an aqueous solution through a tube intragastrically (IG) in a volume of 0.1 ml per 10 g of body weight of the animal in a prophylactic mode 30 minutes before CT, as is customary in the study of frigoprotective properties [2]. Animals were randomly divided into 6 groups: Group 1 – control pathology (CP), purified water 0.1 ml/10 g of body weight i/g+CT (n=6); Group 2 – ASA, 50 mg/kg+CT (n=6); Group 3 – celecoxib, 74 mg/kg+CT (n=6); Group 4 – diclofenac sodium, 14 mg/kg+CT (n=6); Group 5 – montelukast, 1 mg/kg; Group 6 – montelukast, 2 mg/kg. Doses of montelukast were calculated based on average therapeutic human doses using FDA-recommended conversion factors [11]. Taking into account the data [1], the dose of ASA 50 mg/kg was chosen as one that shows a pronounced frigoprotective activity. Doses of celecoxib 74 mg/kg and diclofenac sodium 14 mg/kg are most effective in HT [2].

The anticonvulsant activity of montelukast was studied in a baseline screening model for seizures caused by pentylenetetrazole (PTZ) in 37 adult white nonlinear mice of both sexes weighing 20-22 g according to guidelines [4, 12]. The classic anticonvulsant drug sodium valproate (Depakine, Sanofi-Aventis, France) at an effective dose of 300 mg/kg was chosen as a comparison drug [5, 12, 14]. Animals were randomized into groups: group 1 (CP) – PTZ-induced seizures without drugs (n=12+7, two subgroups were created taking into account the chronopharmacological factor, as sodium valproate and montelukast were studied on different days), purified water 0.1 ml/10g of body weight i/g+PTZ; group 2 – animals with model convulsions, receiving sodium valproate, 300 mg/kg i/g (n=12), group 3 – animals with model convulsions (n=6), which received montelukast at a dose of 4 mg/kg i/g within the range of therapeutic anti-inflammatory dose [8, 13]. In 30 minutes after the studied drugs subcutaneously (s/c) PTZ (Corazol, Sigma, USA) was injected in the form of an aqueous solution at a dose of 90 mg/kg [5, 12, 14]. Each animal was placed in a separate transparent container with a volume of 5 liters and monitored continuously for 60 minutes. Anticonvulsant activity was assessed by the following indicators: latent period of convulsions, the number of clonic-tonic convulsions per 1 animal, percentage of mice with clonic and tonic convulsions, convulsion severity in points (1 point – single tremor, 2 points – "arena" running, 3 points – clonic convulsions, 4 points – clonic-tonic convulsions, 5 points – tonic extension, 6 points – tonic extension, which led to the death of the animal), the duration of the convulsive period, the lifespan of animals to death and mortality. If there were no convulsions for 1hour, it was considered that the latent period is 60 minutes. [4].

Student's T-test in the case of normal distribution was used for statistical processing, in his absence – Mann-Whitney U-test. The analysis was performed using the program Statistica 10.0. (StatSoftInc., Serial No. STA999K347156-W). Differences were considered statistically significant at $p<0.05$. When obtaining the value of $p>0.05$, the difference between the indicators was considered insignificant [3].

The experiments were performed on the basis of the Educational and Scientific Training Center for Medical and Biological Research of the Educational and Scientific Institute of Applied Pharmacy of NUPh in compliance with the principles of the Helsinki Declaration on Humane Treatment of Animals (2000) and the Council Directive which are used for scientific purposes (2010).

The work was performed within the framework of topics funded by the state budget "Rationale for improving the treatment of polypharmacoresistant epilepsy through the combined use of classic anticonvulsants with other drugs" and "Experimental rationale for improving prevention and treatment of cold traumas" (Order of the Ministry of Health of Ukraine No. 2651 of 17.11.2020).

RESULTS AND DISCUSSION

The results of the study of frigoprotective action are shown in Table 1.

According to the results of the study (Table 1), ASA and celecoxib showed a moderate but statistically significant (p=0.044 and p=0.048, respectively) positive effect on life expectancy, increasing it against the indicator of CP group by 16% and 18% on average values and by 26% and 21% on median values. Diclofenac sodium showed greater frigoprotective activity, significantly (p=0.026) increasing the life expectancy of animals by 31% on average and 29% on median value.

Table 1

Effect of montelukast, acetylsalicylic acid, celecoxib and diclofenac sodium on the lifespan of mice with acute cold trauma (M±m; M [Q_{25}; Q_{75}])

Group of animals, drug	Dose, mg/kg	Time, min.		Difference with control (%)	
		M±m	M [Q_{25};Q_{75}]	M±m	M [Q_{25};Q_{75}]
Control pathology	–	44.8±2.5	42 [40;52]	–	–
ASA	50	52.1±1.6*	53 [49;57]*	+16	+26
Celecoxib	74	52.9±2.2*	51 [50;56]*	+18	+21
Diclofenac sodium	14	58.6±4.8*	54 [51;68]*	+31	+29
Montelukast	1	51.7±2.3	51,5 [46;57]	+15	+23
	2	58.7±2.7**	61 [57;62]**	+31	+45

Note. Statistically significant differences with the indicator of the control pathology group: * – p<0.05; ** – p<0.01.

Montelukast showed a dose-dependent frigoprotective effect. At a dose of 1 mg/kg, this effect was in a tendency to increase life expectancy by 15% on average and by 23% on median value. At a dose of 2 mg/kg, high-confidence montelukast (p=0.007) increased the life expectancy of animals by 31% on average and by 45% on median value relative to the CP group. In this effect, montelukast at a dose of 2 mg/kg outweighed the known frigoprotectors with a proven effect of ASA and celecoxib, not inferior to diclofenac sodium at a dose of 14 mg/kg. Blockade of CysLT1-leukotriene receptors is a previously unknown approach to increase the body's resistance to cold.

The results of the study of the anticonvulsant activity of sodium valproate are shown in Table 2.

Sodium valproate at a dose of 300 mg/kg showed a typical pronounced anticonvulsant activity: significantly relative to the indicators of the CP group, it reduced the latent period of paroxysms by 6.05 times (34.06±7.87 vs. 5.63±0.76, p=0.004), the number of clonic-tonic convulsions by 2.4 times per mouse (1.42±0.53 vs. 3.42±0.47, p=0.015), the percentage of animals with clonic and tonic convulsions (50% vs. 100%, p=0.0069 and 33.33% vs. 91.67%, p=0.0047), respectively, the severity of paroxysms – by 2.8 times (2.00±0.64 points vs. 5.58±0. 29 points, p=0.0003), the duration of the convulsion period – by 1.9 times (5.10±2.24 minutes vs. 9.68±1.96 minutes, p=0.045) and mortality – by 10 times (8.33 vs. 83.33, p=0.0003).

The results of the effect of montelukast on the course of convulsions in mice are shown in Table 3.

As can be seen from Table 3, montelukast tended to reduce the number of clonic-tonic convulsions per animal, the proportion of animals with tonic paroxysms, and the severity of convulsions. Montelukast statistically significantly reduced the integral rate of seizure – animal mortality – by 2.57 times (85.71% vs. 33.33%, p=0.042) relative to the CP group. According to this indicator, it has no statistically significant differences from the comparison drug – sodium valproate, which ensured the survival of 8.33% of animals.

Table 2

The effect of sodium valproate on the course of PTZ-induced convulsions in mice (M±m)

Indicators	CP (PTZ, 90 mg/kg), n=12	Sodium valproate (300 mg/kg) + PTZ (90 mg/kg), n=12
Latent period, min.	**5.63±0.76**	**34.06±7.87****
The number of clonic-tonic convulsions per 1 mouse	**3.42±0.47**	**1.42±0.53***
% of mice with convulsions		
clonic	**100**	**50****
tonic	**91.67**	**33.33****
Severity of convulsions, points	**5.58±0.29**	**2.00±0.64****
Duration of the convulsion period, min.	**9.68±1.96**	**5.10±2.24***
Life span of animals before death, min.	**15.71±2.70**	**20.9**
Lethality,%	**83.33**	**8.33****

Note. Statistically significant differences with the indicators of the control pathology group: * – $p<0.05$; ** – $p<0.01$.

In our study of anticonvulsant activity of a single montelukast injection a dose of 4 mg/kg was used, being a conditionally therapeutic anti-inflammatory dose. The anticonvulsant properties of montelukast have previously been studied [15, 16] in doses significantly higher than ours. In a model of PTZ-kindling in mice, this drug at a dose of 10 mg/kg increased the latent period of convulsions [16]. In rats with PTZ-induced convulsions, montelukast had an anticonvulsant effect, dose-dependently reducing lipid peroxidation and increasing brain superoxide dismutase activity at doses of 25-100 mg/kg [15]. Expressed in terms of mice using the species sensitivity coefficient, these doses correspond to 54-217 mg/kg – by 13-54 times higher than in our study. It is known that chemoinduced convulsion models, including PTZ-induced, activate the synthesis of CysLTs [9]. The results of our study confirm that leukotrienes play an important role in the pathogenesis of convulsive syndrome, and blockade of leukotriene receptors is a very effective way of anticonvulsant action.

Table 3

Influence of montelukast on the course of PTZ-induced convulsions in mice (M±m)

Indicators	CP (PTZ, 90 mg/kg), n=7	Montelukast (4 mg/kg) + PTZ (90 mg/kg), n=6
Latent period, min.	**4.41±0.94**	**4.87±0.61**
The number of clonic-tonic convulsions per 1 mouse	**2.29±0.52**	**1.83±0.40**
% of mice with convulsions:		
clonic	**100**	**100**
tonic	**85.71**	**66.67**
Severity of convulsions, points	**5.57±0.43**	**4.33±0.56**
Duration of the convulsive period, min.	**9.66±4.34**	**6.49±2.90**
Life span of animals before death, min.	**15.44±4.50**	**9.97±3.54**
Lethality	**85.71**	**33.33***

Note. Statistically significant differences with the group of control pathology: * – $p<0.05$.

Thus, the non-obvious pharmacological properties of the leukotriene receptor blocker montelukast – frigoprotective and anticonvulsant – have been established. They go beyond the well-known indications for the use of this drug – bronchial asthma and allergic rhinitis. These results justify the feasibility of in-depth studies of the mechanisms of frigoprotective and anticonvulsant action of montelukast, which may supplement the list of drugs for the treatment of CT and adjuvant pharmacotherapy of epilepsy, especially in combination with bronchial asthma and allergic rhinitis. In particular, it can be considered promising to find out the effectiveness of montelukast on other models of CT (frostbite), as well as on other models of convulsive syndrome, including resistant to classical anticonvulsants, and in combination with them.

CONCLUSIONS

1. The model of acute cold trauma revealed a powerful frigoprotective effect of montelukast (2 mg/kg), which is not inferior to or superior in this property to the known frigoprotectors - non-selective and selective COX-2 inhibitors.
2. In the model of pentylenetetrazole convulsions montelukast (4 mg/kg) has a moderate anticonvulsant effect.

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

СПИСОК ЛІТЕРАТУРИ

1. Бондарев Є. В. Влияние глюкозамина гидрохлорида и ацетилсалициловой кислоты на показатели углеводного обмена при острой холодовой травме у крыс. *Оңтүстік Қазақстан Мемлекеттік Фармацевтика академиясы Хабаршы*. 2015. Т. 70, № 1. С. 22-30. URL: http://dspace.nuph.edu.ua/handle/123456789/7275

2. Капелька І. Г., Штриголь С. Ю. Скринінгове дослідження фригопротекторних властивостей нестероїдних протизапальних препаратів за гострого загального охолодження. *Фармакологія та лікарська токсикологія.* 2019. Т. 13, № 5. С. 338-343. URL: http://dspace.nuph.edu.ua/handle/123456789/21574

3. Ланг Т. А., Сесик М. Как описывать статистику в медицине: руководство для авторов, редакторов и рецензентов / пер. с англ. под ред. В. П. Леонова. 2-е изд. Москва: Практ. медицина, 2016. 480 с.

4. Руководство по проведению доклинических исследований лекарственных средств. Ч. 1 / под ред. А. Н. Миронова и др. Москва: Гриф и К, 2012. 944 с.

5. Тіазолзаміщені похідні 2,4-тіазолідиндіону, що виявляють антиконвульсантнудію: пат. 137726 Україна: МПК C07D 277/00, A61K 31/425, A61P 25/08. № u 201903066; заявл. 28.03.2019; опубл. 11.11.2019, Бюл. № 21. 6 с.

6. Холодова травма: доклінічне вивчення лікарських препаратів з фригопротекторними властивостями: метод. рекомендації / Є. В. Бондарєв та ін. *НФаУ*, 2018. 35 с.

7. Bigelow M. D. Neural stimulation systems for the control of refractory epilepsy: a review. *Journal of Neuro Engineering and Rehabilitation.* 2018. Vol. 16, No. 1. P. 1-17. DOI: https://doi.org/10.1186/s12984-019-0605-x

8. Characterization of cysteinyl leukotriene-related receptors and their interactions in a mouse model of asthma / H. K. Trinh et al. *Prostaglandins, Leukotrienes and Essential Fatty Acids.* 2019. Vol. 141. P. 17-23. DOI: https://doi.org/10.1016/j.plefa.2018.12.002

9. Cysteinyl leukotriene receptor (CysLT) antagonists decrease pentylenetetrazol-induced seizures and blood-brain barrier dysfunction / Q. F. Lenz et al. *Neuroscience*. 2014. Vol. 277. P. 859-871. DOI: https://doi.org/10.1016/j.neuroscience.2014.07.058

10. Epidemiological profile of epilepsy in low income populations / C. A. Espinosa Jovel et al. *Seizure*. 2018. Vol. 56. P. 67-72. DOI: https://doi.org/10.1016/j.seizure.2018.02.002

11. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. URL: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.html

12. Hock F. J. Drug Discovery and Evaluation: Pharmacological Assays / Ed. F. J. Hock. *Springer International Publishing.* 2016. 4314 p. DOI: https://doi.org/10.1007/978-3-319-05392-9

13. LTB4 and montelukast in transplantation-related bronchiolitis obliterans in rats / Z.-L. Tu et al. *Journal of Cardiothoracic Surgery*. 2017. Vol. 12, No. 1. DOI: https://doi.org/10.1186/s13019-017-0605-5

14. Mishchenko M., Shtrygol S., Kaminskyy D., Lesyk R. Thiazole-Bearing 4-Thiazolidinones as New-AnticonvulsantAgents. *Sci. Pharm*. 2020. Vol. 88, No. 1. P. 16. DOI: https://doi.org/10.3390/scipharm88010016

15. Montelukast Inhibits Pentylenetetrazol-Induced Seizures in Rats / B. Cevik et al. *Medical Science Monitor.* 2015. Vol. 21. P. 869-874. DOI: https://doi.org/10.12659/MSM.892932

16. Montelukast reduces seizures in pentylenetetrazol-kindled mice / J. Fleck et al. *Brazilian Journal of Medical and Biological Research.* 2016. Vol. 49, No. 4. P. 1-7. DOI: https://doi.org/10.1590/1414-431X20155031

17. Moz.gov.ua. Як уникнути переохолодження: нагадуємо основні правила поведінки у холод. 2019. URL: https://moz.gov.ua/article/news/jak-uniknuti-pereoholodzhennja-nagaduemo-osnovni-pravila-povedinki-u-holod

18. The Role of Leukotrienes Inhibitors in the Management of Chronic Inflammatory Diseases / D. Meshram et al. *Recent Pat Inflam. Al. Drug Disc*. 2020.Vol.14, No.1. P.15-31. DOI: https://doi.org/10.2174/1872213X14666200130095040

19. Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Frostbite: 2019 Update / S. McIntosh et al. *Wilderness & Environmental Medicine*. 2019. Vol. 4S, No. 30. P. 19-32. DOI: https://doi.org/10.1016/j.wem.2019.05.002

REFERENCES

1. Bondaryev YeV. [Effect of glucosamine hydrochloride and acetylsalicylic acid on carbohydrate metabolism in acute cold injury]. Republican Scientific Journal "Vestnik". 2015;1(70):22-27. Russian. Available from: http://dspace.nuph.edu.ua/handle/123456789/7275

2. Kapelka IG, Strygol SYu. [The comparative research of frigoprotective properties of nonsteroidal anti-inflammatory drugs on the model of acute general cooling]. Farmakologiya ta likarska toksikologiya. 2019;13(5):338-43. Ukrainian. Available from: http://dspace.nuph.edu.ua/handle/123456789/21574

3. Lang TA. [How to describe statistics in medicine. Guide for authors, editors and reviewers]. 2nd ed. editor Leonova VP, Moskva: Prakticheskaya meditsina; 2016. p. 480. Russian.

4. Mironov AN, Bunyatyan ND, Vasileva AN., editors. [Guidelines for conducting pre-clinical trials of medicines. Part one]. Moskva: Grif and K.; 2012, p. 944. Russian.

5. Mishchenko MV, Strygol SYu, Lesyk RB, Kaminskyy DV., inventors. National University of Pharmacy, assignee. [Thiazole substituted 2,4-thiazolidinedione derivatives reveal anticonvulsant activity]. Ukrainian patent UA 137726. 2019 Nov 11. Ukrainian.

6. Bondaryev YeV., et al. [Pleclinical sudy of drugs with frigiprotective properties: guidlines]. Kharkiv: Nuph. 2018. Ukrainian.

7. Bigelow MD, Kouzani AZ. Neural stimulation systems for the control of refractory epilepsy: a review. Journal of Neuro Engineering and Rehabilitation. 2018;16(1):1-17. doi: https://doi.org/10.1186/s12984-019-0605-x

8. Trinh HKT, Suh D-H, Nguyen TVT, Choi Y, Park H-S, Shin YS. Characterization of cysteinyl leukotriene-related receptors and their interactions in a mouse model of asthma. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2019;141:17-23. doi: https://doi.org/10.1016/j.plefa.2018.12.002

9. Lenz QF, Arroyo DS, Temp FR, Poersch AB, Masson CJ, Jesse AC., et al. Cysteinyl leukotriene receptor (CysLT) antagonists decrease pentylenetetrazol-induced seizures and blood-brain barrier dysfunction. Neuroscience. 2014;277:859-71. doi: https://doi.org/10.1016/j.neuroscience.2014.07.058

10. Espinosa Jovel CA, Ángel Aledo-Serrano, Rafael Toledano, Antonio Gil-Nagel, Irene García-Morales. Epidemiological profile of epilepsy in low income populations. Seizure. 2018;56:67-72. doi: https://doi.org/10.1016/j.seizure.2018.02.002

11. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. [Internet]. URL: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.html.

12. Hock FJ. Drug Discovery and Evaluation: Pharmacological Assays. Editor FJ Hock. Springer International Publishing; 2016. p. 4314. doi: https://doi.org/10.1007/978-3-319-05392-9

13. Tu Z-L, Zhou Z-Y, Xu H-C, Cao J-L, Ye P, Wang L-M. LTB4 and montelukast in transplantation-related bronchiolitis obliterans in rats. Journal of Cardiothoracic Surgery. 2017;12(1). doi: https://doi.org/10.1186/s13019-017-0605-5

14. Mishchenko M, Shtrygol S, Kaminskyy D, Lesyk R. Thiazole-Bearing 4-Thiazolidinones as New Anticonvulsant Agents. Sci. Pharm. 2020;88(1):16. doi: https://doi.org/10.3390/scipharm88010016

15. Cevik B, Solmaz V, Aksoy D, Erbas O. Montelukast Inhibits Pentylenetetrazol-Induced Seizures in Rats. Medical Science Monitor [Internet]. 2015(21):869-874. doi: https://doi.org/10.12659/MSM.892932

16. Fleck J, Temp F, Marafiga J, Jesse A, Milanesi L, Rambo L. Montelukast reduces seizures in pentylenetetrazol-kindled mice. Brazilian Journal of Medical and Biological Research [Internet]. 2016; 4(49):1-7. doi: https://doi.org/10.1590/1414-431X20155031

17. [How to avoid hypothermia: recall the basic rules of behavior in the cold]. [Internet]; 2019. Ukrainian. Available from: https://moz.gov.ua/article/news/jak-uniknuti-pereoholodzhennja-nagaduemo-osnovni-pravila-povedinki-u-holod

18. Meshram D, Bhardwaj K, Rathod C, Mahady GB, Soni KK. The Role of Leukotrienes Inhibitors in the Management of Chronic Inflammatory Diseases. Recent Pat Inflamm Allergy Drug Discov. 2020;14(1):15-31. doi: https://doi.org/10.2174/1872213X14666200130095040

19. McIntosh S, Freer L, Grissom C, Auerbach P, Rodway G, Cochran A. Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Frostbite: 2019 Update. Wilderness & Environmental Medicine [Internet]. 20194S(30):19-32. doi: https://doi.org/10.1016/j.wem.2019.05.002

The article was received
2020.05.29