Department of Pharmacology and Pharmacotherapy[1], Department of Analytical Chemistry and Analytical Toxicology[2], Educational and Scientific Institute of Applied Pharmacy[3], National University of Pharmacy, Kharkiv, Ukraine

Effect of arachidonic acid cascade inhibitors on body temperature and cognitive functions in rats in the Morris water maze after acute cold injury

I.Kapelka[1,*], S. Shtrygol'[1], O. Koiro[1], S. Merzlikin[2], O. Kudina[1], T. Yudkevych[3]

Received April 28, 2021, accepted May 7, 2021

**Corresponding author: Ihor Kapelka, Department of Pharmacology and Pharmacotherapy, National University of Pharmacy, Kharkiv, 53 Pushkinska str., 61002, Ukraine*
kig1997@gmail.com

Pharmazie 76: 313-316 (2021) *doi: 10. 1691/ph.2021.1571*

Objective: The aim of the study was to evaluate the effect of arachidonic acid cascade inhibitors on body temperature and cognitive functions of rats (spatial memory, learning ability) in the Morris water maze test (MWM) after acute cold injury (CI). *Methods:* Animals were trained to find an escape platform in the MWM for two consecutive days. On the third day, rats were treated with saline (10 ml/kg), diclofenac sodium (7 mg/kg), etoricoxib (5 mg/kg), darbufelone mesylate (20 mg/kg) or montelukast (1 mg/kg) intragastrically (i.g.), 30 minutes before CI modeling. Air hypothermia with an acute general cooling (AGC) model was used as a kind of CI. Animals were subjected to cooling for 2 hours at -18°C. Body temperature was measured before and after CI modeling. MWM experimental trials tests were carried out after cold exposure. Number of successful trials, escape latency, distance moved, velocity, meander, and behavioral patterns in individual quadrants were recorded. *Results:* In the control pathology group, a statistically significantly body temperature decrease was observed ($p<0.05$ compared to the initial value). All of the studied drugs reduced hypothermia severity, but only in the sodium diclofenac group this reduction reached a significant level in comparison with the untreated animals ($p<0.01$). A tendency to reduce the severity of hypothermia was observed in the group of animals treated by etoricoxib, darbufelone mesylate, and montelukast. In the control pathology group, the number of successful trials was significantly decreased ($p<0.01$), velocity ($p<0.05$), and escape latency ($p<0.05$) were increased compared with intact animals. Diclofenac sodium significantly reduced escape latency ($p<0.05$) and increased the number of successful trials in comparison with the control pathology group ($p<0.01$). Montelukast tended to improve, etoricoxib and darbufelone mesylate did not improve cognitive functions of rats with CI. *Conclusions:* The results experimentally substantiate the possibility of effective pharmacoprophylaxis of CI and its negative effects on cognitive functions while applying arachidonic acid cascade inhibitors, particularly the non-selective COX inhibitor diclofenac sodium.

1. Introduction

Cold is one of the environmental factors that constantly threaten human health and life. Athletes, occupational workers, and military personnel are often required to work in cold environments (Jones et al. 2017). Cold injuries (CI) are of significant military concern because of their adverse impact on operations and the high financial costs of treatment and disability (Paton 2001). For example, from 2018 to 2019, the incidence of CI in the U.S. Army was 36.5 cases per 100,000 people (U.S. Armed Forces 2019).

Excessive exposure to low temperature causes localized injury, systemic hypothermia, or a combination of both (Long et al. 2005). Cold is a disease-producing factor for all organs and systems of the body. Products of the arachidonic acid metabolism, namely prostaglandin $F_2\alpha$ ($PGF_2\alpha$), thromboxane A_2 (TXA_2), and leukotrienes, play an important role in the pathogenesis of CI. $PGF_2\alpha$ and TXA_2 enhance platelet aggregation and vasoconstriction, which results in the formation of blood clots in vasculature and ischemia. Cysteinyl leukotrienes not only modulate the inflammatory process but also play a significant role in the development of brain cryoinjury (Ding et al. 2007; McIntosh et al. 2019). In addition to direct pathological effects, cold provokes powerful polymodal stress and disconnects the cerebral cortex functioning (Kochkin et al. 2017). Acute exposure to cold has been shown to cause cognitive impairment, decrease of attention, reaction speed, short-term memory, and overall mood (Jones et al. 2017; Lieberman et al. 2009; Solianik et al. 2014). This is particularly dangerous during combat missions or in case of performing complex responsible tasks. Therefore, the scientific inquiry designed to find the various approaches of CI pharmacological correction on cognitive functions continues. Previous studies have shown that the use of tyrosine for 30 min before cold exposure has a beneficial effect on working memory in humans (Mahoney et al. 2007). Another promising remedy is an *Empetrum nigrum* extract. The results of the Morris water maze test in rats having been exposed to cold stress have shown that administration of the extract for 10 days increases stress tolerance and improves cognitive abilities (Kochkin et al. 2017).

Considering the role of prostaglandins and leukotrienes in the CI pathogenesis, the administration of arachidonic acid cascade inhibitors may be a promising approach in prevention of cold traumas. Our previous screening studies demonstrated the ability of these drugs to increase the life expectancy of animals during cold exposure. Non-selective COX inhibitors (acetylsalicylic acid, ibuprofen, mefenamic acid, diclofenac sodium); moderately selective COX-2 inhibitor (meloxicam); highly selective COX-2 inhibitors (celecoxib, etoricoxib), dual COX-2/5-LOX inhibitors (darbufelone, darbufelone methansulfonate (mesylate), 5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)2-(thiazole-2-il-imino)-thiazolidine-4-on), COX-3 inhibitor (paracetamol); CysLT1-leukotriene receptor blocker (montelukast) were tested at 2-3 doses as potential frigoprotectors. Among these twelve arachi-

donic acid cascade inhibitors with different mechanisms of action, the leaders were diclofenac sodium, etoricoxib, darbufelone mesylate, and montelukast. They increased the life expectancy of mice at -18 °C by 30-65% (Kapelka and Shtrygol' 2019; Kapelka et al. 2020).

However, the effect of the arachidonic acid cascade inhibitors on the central nervous system, whose damage plays a significant role in the pathogenesis and prognosis of CI progression, remains unknown. Therefore, the aim of this study is to evaluate the effect of the arachidonic acid cascade inhibitors with different mechanisms of action on the hypothermia progression and cognitive functions of rats (spatial memory, learning ability) after CI in the MWM test.

2. Investigations and results

2.1. Antihypothermic action of arachidonic acid cascade inhibitors after CI

Cold exposure caused a statistically significant decrease of the rats' body temperature ($p<0.05$), but all the studied drugs reduced the hypothermia severity to varying degrees (Table 1).

Table 1: Changes of rats' body temperature after acute general cooling when applying arachidonic acid cascade inhibitors before testing in the Morris water maze, M±m; Me[Q25; Q75]

Group, number of animals	Body temperature, °C		
	Initial	After cold exposure	Difference
Vehicle control, n=7	37.0±0.1 36.9[36.7;37.1]	–	–
Control pathology – AGC, n=7	36.9±0.1 36.9[36.8;37.0]	35.6±0.2[a] 35.2[35.0;36.2][a]	–1.4±0.3 –1.3[–1.9;–0.8]
AGC + diclofenac sodium, n=7	37.6±0.3 37.8[36.9;38.1]	37.6±0.2 37.7[37.0;38.0]	0.0±0.1[bb] –0.1[–0.1;0.2][bb]
AGC + etoricoxib, n=7	37.6±0.1 37.7[37.5;37.8]	36.7±0.3[a] 36.7[36.2;37.0][a]	–1.0±0.3[c] –1.0[–1.5;–0.1][c]
AGC + darbufelone mesylate, n=7	37.3±0.1 37.3[37.2;37.4]	36.4±0.2[a] 36.5[35.9;36.8][a]	–0.9±0.2[cc] –0.7[–1.5;–0.5][cc]
AGC + montelukast, n=7	37.5±0.2 37.6[36.9;37.8]	36.6±0.3[a] 36.5[35.9;37.4][a]	–0.9±0.3[c] –0.9[–1.7;–0.8][c]

Notes. AGC – acute general cooling. Statistically significant differences: a – $p<0.05$ compared to the initial within the group; b – $p<0.05$ compared to the control pathology group; bb – $p<0.01$ compared to the control pathology group; c – $p<0.05$ compared to the diclofenac sodium group; cc – $p<0.01$ compared to the diclofenac sodium group.

In the control pathology group, a significant ($p<0.05$) decrease in the rats' body temperature by an average of 1.4°C was observed. In the diclofenac sodium group, no significant temperature changes occurred, therefore, this drug prevented the development of hypothermia ($p<0.01$). There was a tendency to reduce the severity of hypothermia in the darbufelone mesylate, etoricoxib, and montelukast groups.

2.2. Memory and learning performance in the MWM

The platform search parameters in the MWM are presented in the Table 2. In the MWM test, animals of the control pathology group searched for the platform more than 2 times longer than the intact rats, with an average escape latency of 138.0 s versus 65.8 s, respectively ($p<0.05$). The longer escape latency might be explained by a decrease in rats' velocity in the control pathology group: it was 12.4 cm/s which was significantly ($p<0.05$) lower than in the vehicle control group (22.1 cm/s). The number of successful trials in the control pathology group was less than in the vehicle control – 57% vs. 100% ($p<0.01$). The distance and the meander also tended to increase, which indicated less confidence in movement direction during the platform searching.

Diclofenac sodium had a positive effect on the rats' cognitive functions after CI. The effect of this non-selective COX inhibitor was accompanied with a significant ($p<0.05$) reduction of escape latency by almost 2 times compared to the control pathology group. At the same time, the difference in the velocity of animals under the influence of diclofenac sodium, unlike the other studied drugs, did not reach significant values compared to the vehicle control group. The number of successful trials was equal to the vehicle control group (100% of animals) and significantly higher than in the control pathology group ($p<0.01$). There was a tendency to reduce the meander and the distance in comparison with the control pathology.

Etoricoxib and darbufelone mesylate did not improve spatial orientation and learning efficiency after CI: the number of successful trials and all platform search parameters in the MWM were at the same level as in the control pathology group, while the velocity was significantly lower than in the intact control group ($p<0.05$). Montelukast tended to increase the number of successful trials by up to 86%, but did not have a significant positive effect on the other parameters. The influence of the leukotriene receptor blocker resulted in significantly higher velocity of the rats compared to the etoricoxib group ($p<0.05$), but statistically significantly lower than the corresponding parameter of the intact animals ($p<0.05$).

Table 2: Effect of CI and arachidonic acid cascade inhibitors on spatial memory and learning efficiency of rats in the Morris Water Maze, M±m; Me[Q25; Q75]

Group	Parameters				
	Number of successful trials, ABS./%	Escape latency, s	Distance moved, cm	Velocity, cm/s	Meander, degree/cm
Vehicle control, n=7	7/100	65.8±22.4 70[15;96]	1213.7±348.6 1141[332;2271]	22.1±2.8 21[16;30]	33.4±16.1 9.7[6.1;79.1]
Control pathology – AGC, n=7	4/57[aa]	138.0±17.9[a] 143[107;180][a]	1681.3±224.5 1623[1195;2186]	12.4±0.7[a] 13[10;14][a]	62.9±42.4 25.3[13.7;30.4]
AGC + diclofenac sodium, n=7	7/100[bb]	81.4±12.5[b] 72[68;97][b]	1436.6±436.6 1122[536;2187]	16.9±3.8 16[12;17]	23.4±6.7 17.0[12.6;42.3]
AGC + etoricoxib, n=7	4/57[aa]	116.9±22.7 91[59;180]	1233.9±332.0 947[586;1835]	11.2±0.9[aa] 10[9;14][aa]	97.9±67.9 27.1[11.5;83.4]
AGC + darbufelone mesylate, n=7	4/57[aa]	119.6±23.7 110[67;180]	1678.1±425.3 1481[921;2632]	13.0±1.4[a] 14[11;15][a]	20.0±7.9 12.9[9.4;20.4]
AGC + montelukast, n=7	6/86	127.9±11.8[ac] 115[98;148][ac]	1934.8±242.8 1763[1323;2358]	15.0±1.0[ad] 16[12;17][ad]	63.0±22.0 45.0[15.9;115.9]

Notes. AGC – acute general cooling. Statistically significant differences: a – $p<0.05$ compared to the vehicle control group; aa – $p<0.01$ compared to the vehicle control group; b – $p<0.05$ compared to the control pathology group; bb – $p< 0.01$ compared to the control pathology group; c – $p<0.05$ compared to the diclofenac sodium group; cc – $p<0.01$ compared to the diclofenac sodium group; d – $p<0.05$ compared to the etoricoxib group; dd – $p<0.01$ to the etoricoxib group.

2.3. Inter-quadrant analysis of experimental trials in the MWM

The results of the inter-quadrant analysis of the animals' behavior are presented in Table 3. No significant inter-quadrant behavioral patterns were found in the vehicle control group. The rats of the control pathology group spent significantly more time in the starting quadrant (NE) compared to the target quadrant (SW): 35.0% vs. 17.8%, respectively ($p<0.05$). This could indicate animals disorientation in CI conditions and uncertainty about movement direction. A similar situation for the starting and target quadrants was observed in the diclofenac sodium, darbufelone mesylate, and etoricoxib groups. Only the rats treated with montelukast spent more time in the target quadrant (SW) compared to the starting one (NE) (30.1% vs. 27.0%, respectively), but the difference did not reach the significant values. According to the number of statistically significant inter-quadrant differences, the studied drugs are arranged in the following sequence: etoricoxib > darbufelone mesylate = montelukast > diclofenac sodium.

Table 3. Effect of cold injury and arachidonic acid cascade inhibitors on rats' behavior in separate quadrants of the Morris water maze; M±m; Me[Q25; Q75]

Group	Parameters	Quadrant			
		SW	SE	NW	NE
Vehicle control, n=7	Time spent, s	8.8±1.5 9.5[5.5;11.3]	16.6±5.1 19.5[4.5;26.3]	9.5±2.2 10.5[3.3;14.8]	28.3±13.3 20.2[3.0;35.8]
	Percentage of time, %	23.6±7.1 14.9[14.1;36.3]	26.6±4.8 27.4[20.6;37.4]	19.9±3.6 17.0[13.8;28.6]	33.0±5.2 28.8[23.1;41.5]
	Frequency of appearance	3.6±0.7 4.0[2.0;5.0]	5.9±1.7 6.0[1.0;10.0]	3.6±0.9 3.0[1.0;6.0]	6.7±2.2 8.0[1.0;10.0]
	Average duration of one entry, s	2.6±0.4 2.3[1.8;2.8]	2.7±0.6 2.8[1.9;4.4]	3.1±0.6 2.7[2.0;5.0]	3.4±0.52 3.0[2.5;4.0]
Control pathology – AGC, n=7	Time spent, s	24.6±6.7 17.7[11.0;41.2]	34.2±6.1 33.9[17.5;39.6]	31.1±7.3 32.6[14.4;48.5]	47.3±6.3 53.3[29.4;61.0] [a]**NW**
	Percentage of time, %	17.8±3.5 15.3[9.8;28.8]	26.0±3.5 30.2[20.0;33.1]	20.9±2.9 20.2[13.4;26.9]	35.0±2.9 36.1[31.7;41.5] [a]**SW,** [a]**NW**
	Frequency of appearance	6.0±1.6 4.0[3.0;10.0]	8.3±1.4 8.0[7.0;10.0]	8.4±1.6 9.0[5.0;13.0]	8.1±1.5 7.0[6.0;11.0]
	Average duration of one entry, s	4.2±0.3 4.1[3.6;4.1]	4.2±0.4 4.3[3.6;5.0]	3.5±0.2 3.5[2.9;3.6]	6.2±0.6 6.4[4.8;7.6] [a]**SW,**[a]**SE,** [a]**NW**
AGC + diclofenac sodium, n=7	Time spent, s	13.6±2.4 14.4[7.3;16.3]	15.3±3.5 14.5[13.0;17.0]	17.0±2.9 16.3[10.1;21.6]	24.9±4.3 22.1[17.9;29.6] [a]**SW**
	Percentage of time, %	19.5±2.3 22.1[19.4;22.6]	20.0±2.9 20.7[14.6;23.5]	24.4±3.2 27.7[15.4;29.9]	35.6±4.3 34.7[25.5;45.1] [a]**SW**
	Frequency of appearance	4.5±0.9 4.0[3.0;6.0]	5.0±1.9 4.0[3.0;4.0]	5.2±1.1 4.5[3.0;8.0]	5.5±0.9 5.0[4.0;5.0]
	Average duration of one entry, s	3.2±0.5 3.0[2.5;3.6]	3.6±0.4 3.3[3.1;4.3]	3.7±0.6 3.9[2.7;4.7]	4.8±0.9 4.2[3.6;5.9]
AGC + etoricoxib, n=7	Time spent, s	14.4±2.2 14.6[10.4;19.0]	30.3±8.4 24.8[14.2;42.3] [a]**SW**	16.3±0.9 16.8[15.3;17.7] [a]**SW**	43.3±13.8 28.2[21.0;66.2] [a]**NW**
	Percentage of time, %	15.2±2.3 14.9[11.3;20.8]	27.3±2.6 26.1[23.5;33.1] [a]**SW**	18.7±3.5 19.3[9.9;21.7]	38.1±5.6 33.3[27.4;53.4] [a]**SW,** [a]**NW**
	Frequency of appearance	4.7±0.8 5.0[3.0;6.0]	7.3±1.3 7.0[6.0;8.0] [a]**SW**	4.5±0.8 4.5[3.0;6.0] [a]**SE**	8.2±2.0 6.5[5.0;10.0] [a]**SW,** [a]**NW**
	Average duration of one entry, s	3.3±0.4 2.9[2.5;3.8]	4.1±0.9 3.5[2.8;4.3]	4.3±1.0 3.5[3.0;4.1]	5.1±0.8 5.1[3.0;6.6]
AGC + darbufelone mesylate, n=7	Time spent, s	21.5±6.7 15.3[12.1;34.0]	31.1±7.8 32.8[13.6;39.4]	22.1±5.0 21.0[12.1;22.3]	43.6±8.4 51.9[17.9;65.2] [a]**SW,**[a]**SE**
	Percentage of time, %	15.6±3.6 14.5[11.0;26.0]	22.8±4.4 21.9[20.4;30.8]	23.5±5.7 21.6[9.9;33.6]	38.1±3.7 36.2[28.8;50.3] [a]**SW,** [a]**SE**
	Frequency of appearance	5.4±1.8 3.0[2.0;11.0]	6.4±1.7 7.0[2.0;9.0]	5.3±1.6 4.0[3.0;6.0]	9.1±2.1 9.0[4.0;15.0] [a]**SW,** [a]**SE**
	Average duration of one entry, s	4.4±0.5 4.6[3.1;5.1]	5.3±0.8 4.9[4.1;6.8]	5.4±1.2 3.7[3.5;7.0]	5.9±1.1 4.5[3.8;6.6]
AGC + montelukast, n=7	Time spent, s	39.1±3.0 35.1[30.5;39.2]	39.5±6.1 38.8[26.7;42.0]	20.0±5.4 19.3[13.1;31.5] [a]**SW,** [a]**SE**	34.9±7.1 26.4[25.1;38.5] [a]**NW**
	Percentage of time, %	30.1±3.2 28.6[24.3;35.0]	32.6±5.3 28.0[25.0;34.8]	15.5±3.5 17.4[13.3;21.3] [a]**SW,** [a]**SE**	27.0±2.3 25.5[23.1;26.7] [a]**NW**
	Frequency of appearance	9.2±1.7 9.5[6.0;1.0]	9.3±2.0 8.5[6.0;11.0]	6.8±2.4 5.0[4.0;10.0]	11.2±2.2 10.5[9.0;16.0]
	Average duration of one entry, s	4.6±0.9 4.2[3.1;5.1]	5.0±1.0 4.1[3.6;6.1]	3.3±0.3 3.3[3.2;3.8]	3.8±1.0 2.8[2.2;4.3]

Notes. AGC – acute general cooling. NE – starting quadrant, SW – target quadrants. Statistically significant differences: a – $p<0.05$ compared to the result of this group in the other quadrant.

3. Discussion

All of the studied drugs decreased the hypothermia severity. These results are consistent with the previous studies of the frigoprotective properties (Kapelka and Shtrygol' 2019; Kapelka et al. 2020). But complete prevention of the rats' body temperature decrease was marked in diclofenac sodium among these medicines.

The results of the MWM test prove that CI impairs the animals' cognitive functions. These data are consistent with the results of the study (Kochkin et al. 2017), despite different CI modeling methods – air hypothermia in our study and water immersion in the cited one. Diclofenac sodium improved the main parameters of the MWM test – escape latency, velocity and meander. This means that diclofenac sodium improves both the physical condition and cognitive abilities under hypothermia, and indicates a protective effect of the drug in cold stress. It is possible that this effect is associated particularly with non-selectivity of action – inhibition of both COX-1 and COX-2 that maintains a certain balance between prostaglandins of different classes. At the same time, previous studies have shown a dissociation between the frigoprotective (antihypothermic) and anti-inflammatory properties of diclofenac sodium in a model of carrageenan edema in mice at low ambient temperature (Kapelka and Shtrygol' 2020). This may indicate the existence of separate mechanisms of the frigoprotective action of non-steroidal anti-inflammatory drugs, but requires special clarification of the arachidonic acid cascade state in such conditions, which will be the subject of our further research.

Darbufelone mesylate and etoricoxib did not demonstrate significant activity in the current study. Selective COX-2 inhibitors are associated with an increased risk of thrombosis: these drugs reduce the prostacyclin synthesis, which causes vasodilation and improves the rheological properties of blood. Inhibition of the prostacyclin synthesis disrupts the prothrombotic-antithrombotic balance (Schjerning et al. 2020). This may disturb the cerebral circulation, which can even worse cognitive functions in CI. However, further research is needed to confirm or disprove this assumption.

Montelukast tended to improve the condition of animals, increasing the number of successful trials. This may be explained by the role of leukotrienes in the development of brain cryotrauma (Ding et al. 2007).

The obtained results experimentally substantiate the possibility of reducing the severity of CI by using the arachidonic acid cascade inhibitors (primarily diclofenac sodium) both in relation to the degree of hypothermia and in relation to cognitive functions during cold stress. As a frigoprotector, the non-selective COX inhibitor diclofenac sodium has advantages over the selective COX-2 (etoricoxib) and COX-2/5-LOX (darbufelone mesylate) inhibitors, as well as over the CysLT1-leukotriene receptor blocker montelukast.

4. Experimental

4.1. Animals

Adult (9-month-old) random-bred male rats (249±5 g) were taken from the vivarium of the Central Research Laboratory (National University of Pharmacy, Kharkiv, Ukraine). The animals were kept in standard polypropylene cages, at 20-26°C and 50% humidity in the well-ventilated room with a 12-hour light/dark cycle and free access to food and water. The work was carried out in the Central Research Laboratory of the Educational and Scientific Institute of Applied pharmacy of National University of Pharmacy in compliance with Directive 2010/63/EU of the European Parliament and the Council "On the protection of animals used for scientific purposes" (Brussels, 2010). All experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy.

4.2. Drugs and chemicals

There are substances used for the study: non-selective COX inhibitor diclofenac sodium (Voltaren® tablets, Novartis, Switzerland), highly selective COX-2 inhibitor – etoricoxib (Arcoxia® tablets, Merck Sharp&Dohme idea Inc, USA), dual COX-2/5-LOX inhibitor – darbufelone methanesulfonate (mesylate), CysLT1 receptor blocker – montelukast (Singular®, tablets, Merck Sharp&Dohme idea Inc, USA). Darbufelone mesylate was synthesized at the Department of Pharmaceutical, Organic, and Bioorganic Chemistry of the Danylo Galytsky Lviv National Medical University headed by Professor R. Lesyk (Kapelka et al. 2020). The drugs and the darbufelon mesylate substance were grinded and suspended with the addition of Tween-80, administered as an aqueous solution i.g. in a volume of 0.5 ml/100 g of the body weight in a preventive mode for 30 min before cold exposure (Bondariev et al. 2018).

Rats were separated into six groups by random selection:

1. Vehicle control group (n=7) – animals were treated with saline i.g. (10 ml/kg);
2. Control pathology group (n=7) – animals were treated with saline i.g. 30 minutes before cold exposure (10 ml/kg);
3. Group of diclofenac sodium (n=7), which was administered i.g. at a dose of 7 mg/kg;
4. Group of etoricoxib (n=7), which was administered i.g. at a dose of 5 mg/kg;
5. Group of darbufelone mesylate (n=7), which was administered i.g. at a dose of 20 mg/kg;
6. Group of montelukast (n=7), which was administered i.g. at a dose of 1 mg/kg.

Doses of diclofenac sodium, etoricoxib, and darbufelone mesylate were selected based on the results of the screening studies as the most effective in animal life expectancy increase (Kapelka and Shtrygol' 2019; Kapelka et al. 2020). The dose of montelukast was calculated with the cross-species sensitivity coefficient (Nair and Jacob 2016).

4.3. Cold injury model, body temperature control

Acute general cooling was used as a model of air hypothermia. In order to reproduce CI, animals were placed in separate transparent plastic containers with a volume of 5000 cm^3 without restricting motor activity and air access. Containers with rats were placed in the "Nord Inter-300" freezer at a temperature of -18°C for 2 h. Body temperature was measured rectally 5 min before and after the exposure with a Panlab TMP812 RS thermometer (Bondariev et al. 2018).

4.4. Morris water maze

The experiment was carried out in a dimly lit and soundproof test room. The Morris water maze consisted of a circular pool (120 cm in diameter and 55 cm in depth) that was filled with water (25°C) to a depth of 45 cm (Vogel 2008). The pool was conventionally divided into 4 quadrants: Northwest (NW), Northeast (NE), Southeast (SE), and Southwest (SW). On the edge of the pool board, eight landmarks were placed at the same intervals – plastic figures of different colors and shapes. The 9 cm diameter escape platform was placed in a fixed location in the SW quadrant, protruded 1 inch above the water surface level during training, and was 1 inch below the water surface level during testing (Morris 1984; Nunez 2008).

There were two training days. Each animal underwent three training trials every day. During the training trials, three starting positions were used sequentially: North, NE, and East (relative to the center of the circle). The animal was placed in the pool facing the board and had 60 s to search for the platform. If it did not find the platform within 60 s, it was carefully brought to it. On the platform, the animal spent 15 s, after which it was allowed to rest for 60 s and repeated training from the next starting position. On the second day, the training was conducted according to the same algorithm, but the starting positions were used in reverse order: East, NE, and North.

On the third day, CI was modeled. Ten minutes after the end of cold exposure, an experimental trial was performed, during which the water in the pool was made opaque with powdered milk, and the platform was lowered 1 inch below the water surface level. The starting quadrant was NE, and the target quadrant was SW. If the animal did not find the platform in 180 s, it was removed from the pool. The study was recorded with a Trust Viveo HD 720p video camera focused on the entire pool area. Navigation parameters (escape latency, distance, velocity, meander, behavior in individual quadrants) were analyzed using the Noldus EthoVision XT 15 program.

4.5. Statistical analysis

Statistical analysis of the results was performed using the program "Statistica 10.0". The normality hypothesis was rejected by the Shapiro-Wilk test. The comparisons of the central tendencies were made using the Kruskel-Wallis H-test and the Mann-Whitney U-test. Differences between dependent samples were evaluated according to the paired Wilcoxon signed-rank test. The Fisher exact test was used to compare qualitative features. The differences were considered statistically significant at $p<0.05$. Quantitative data were presented as an arithmetic mean with standard error of the mean (M±m), medians with 25% and 75% percentiles ($Me[Q_{25}; Q_{75}]$), and percentages.

Funding: The research was carried out within the framework of the topic "Experimental substantiation for improving the effectiveness of prevention and treatment of cold injuries" of the list of scientific studies of the Ministry of Health of Ukraine, carried out at the expense of the state budget of Ukraine No. 0120u102460 (Order of the Ministry of Health of Ukraine No. 2651 of 17.11.2020).

Conflict of interest: none declared.

References

Bondariev YV, Shtrygol' SY, Drogovoz SM, Shchokina KG (2018) Cold injury: preclinical study of drugs with frigoprotective properties. Guidelines, Kharkiv, p. 35

Ding Q, Fang S, Zhou Y, Zhang L, Zhang W, Chen Z, Wei E (2007) Cysteinyl leukotriene receptor 1 partially mediates brain cryoinjury in mice. Acta Pharmacol Sin 28: 945–952.

Jones DM, Bailey SP, Roelands B, Buono MJ, Meeusen R (2017) Cold acclimation and cognitive performance: A review. Auton Neurosci 208: 36–42.

Kapelka IG, Shtrygol' SY (2020) The comparative research of frigoprotective properties of nonsteroidal anti-inflammatory drugs on the model of acute general cooling. Pharmacol Drug Toxicol 13: 338–343.

Kapelka IG, Shtrygol' SY (2019) The characteristics of the anti-inflammatory action of sodium diclofenac in cold and normal environment. News of Pharmacy 2: 106–112.

Kapelka IG, Shtrygol' SY, Lesyk RB, Lozynskyi AV, Khomyak SV, Novikov VP (2020) The comparative research of arachidonic acid cascade inhibitors for frigiprotective activity. Pharmacology and Drug Toxicology 14: 122–128.

Kochkin RA, Lobanov AA, Andronov SV, Kostricin VV, Popov AA, Lobanova LP, Kobelkova IV, Kambarov AO (2017) Efficiency of black crowberry (Empetrum Nigrum L.) in correction of cold stress. J New Med Technol 4: 66–72.

Lieberman HR, Castellani JW, Young AJ (2009) Cognitive function and mood during acute cold stress after extended military training and recovery. Aviat Space Environ Med 7: 629–636.

Long WB, Edlich RF, Winters KL, Britt LD (2005) Cold injuries. J Long Term Eff Med Implants 15: 67–78.

Mahoney CR, Castellani J, Kramer FM, Young A, Lieberman HR (2007) Tyrosine supplementation mitigates working memory decrements during cold exposure. Physiol Behav 92: 575–582.

McIntosh SE, Freer L, Grissom CK, Auerbach PS, Rodway GW, Cochran A, Giesbrecht GG, McDevitt M, Imray CH, Johnson EL, Pandey P, Dow J, Hackett PH (2019) Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Frostbite: 2019 Update. Wilderness Environ Med 30: 19–32.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11: 47–60.

Nair AB, Jacob S (2016) A simple practice guide for dose conversation between animals and human. J Basic Clin Pharm 7: 27–31.

Nunez J (2008) Morris Water Maze Experiment. J Vis Exp e897: 1–3.

Paton BC (2001) Cold, casualties, and conquests: the effects of cold on warfare. In: Pandolf KB, Burr RE (ed). Medical aspects of harsh environments, Volume 1., Falls Church, VA, p. 313–349.

Schjerning AM, McGettigan P, Gislason G (2020) Cardiovascular effects and safety of (non-aspirin) NSAIDs. Nat Rev Cardiol 17: 574–584.

Solianik R, Skurvydas A, Mickeviciene D, Brazaitis M (2014) Intermittent whole-body cold immersion induces similar thermal stress but different motor and cognitive responses between males and females. Cryobiology 69: 323–332

Vogel HG (2008) Drug Discovery and Evaluation: Pharmacological Assays (3rd edn). Springer, Berlin, Heidelberg, New York, p. 915–917.

U.S. Armed Forces (2019) Update: Cold weather injuries, active and reserve components, July 2014-June 2019. MSMR 26:17-26