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THE INFLUENCE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS WITH DIFFERENT MECHANISMS OF ACTION ON THE COURSE OF STRESS REACTION, THE FUNCTIONAL STATE OF KIDNEYS, LIVER, AND HEART ON THE MODEL OF ACUTE GENERAL COOLING

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Inhibitors of the arachidonic acid cascade have significant potential as the agents for the prevention of severe cold injuries. The results of the previous studies have demonstrated the pronounced frigoprotective properties of certain non-steroidal anti-inflammatory drugs, primarily diclofenac sodium, etoricoxib, darbufelone mesylate, under the conditions of acute general cooling.

The aim *of the study: to investigate the effect of non-steroidal anti-inflammatory drugs with various mechanisms of action on the course of the stress reaction, the functional state of the kidneys, liver, and heart using the model of acute general cooling.*

Materials and Methods. *The experiment was carried out using 35 outbreed male rats weighing 256±5 g. The studied drugs were administered intragastrically 30 minutes before cold injury modelling: diclofenac sodium at a dose of 7 mg/kg, etoricoxib at a dose of 5 mg/kg, darbufelone mesylate at a dose of 20 mg/kg. Acute general cooling was induced by exposure at –18 °C for 2 hours. The efficacy of the studied drugs was evaluated by the values as follows: the body temperature (measured rectally), the course of a stress reaction according to the criteria of "the stress triad", the functional state of the kidney and liver according to the changes in the blood serum biochemical parameters, the functional state of the heart according to the electrocardiogram.*

Results*. It was found that etoricoxib and darbufelone mesylate, and especially diclofenac sodium, demonstrate frigoprotective properties, reducing the severity of hypothermia, have stress-protective activity and a beneficial effect on the functional state of the kidneys. All investigated non-steroidal anti-inflammatory drugs prevent a decrease in myocardial contractility (by the effect on the systolic index) and lengthening of the QT interval caused by acute cold injury. Diclofenac sodium, unlike etoricoxib and darbufelone mesylate, does not enhance the effect of acute general cooling on intraventricular conduction. Under acute exposure to cold, no significant changes in the functional state of the liver were observed, including the groups receiving the nonsteroidal anti-inflammatory medicines.*

Conclusions*. The prophylactic administration of the arachidonic acid cascade inhibitors, especially the non-selective COX-2 inhibitor diclofenac sodium, reduces the severity of the stress response, contributes to the maintenance of the renal and cardiac function. There are no significant changes in the functional state of the liver under conditions of the experiment*

Keywords*: acute general cooling, sodium diclofenac, etoricoxib, darbufelone mesylate, kidneys, liver, heart, cold stress*

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1. Introduction

Exposure to low temperatures poses a significant threat to human health. However, it is difficult to assess the real prevalence of cold injuries and hypothermia because the information registered by medical institutions is limited data concerning the most severe cases [1]. According to the results released by the U.S. Centers for Disease Control and Prevention, in 2019, the prevalence of hypothermia-related deaths among people aged 15 and older ranged from 0.2 to 8.6 cases per 100,000 of population. Higher rates were recorded in rural areas compared to urban areas, as well as among older people [2].

The human body is regularly exposed to adverse environmental conditions, including cold, heat, ultraviolet radiation, windy weather, and high relative humidity. In particular, the influence of low temperatures significantly affects the functioning of the neuroendocrine [3], cardiovascular [4], respiratory [5] systems, affects immunity [6, 7], metabolic processes [8, 9], the functional state of the kidneys [10], etc.

Acute general cooling (AGC) leads to an increase of stress hormones like cortisol, epinephrine, and norepinephrine in the blood [11, 12]. Stimulation of cold receptors causes the activation of mechanisms providing changes in heat production and heat transfer. The activity of the sympathetic nervous system increases, which results in heart rate changes, vasoconstriction, and an increase in blood pressure [1, 4]. Renal blood flow and

glomerular filtration rate (GFR) are reduced. Nevertheless "cold diuresis" may develop. It is associated with impairment of sodium and water reabsorption in the distal tubules, inhibition of antidiuretic hormone secretion [10, 13]. Acute cold stress could lead to a decrease in the content of glycogen in the liver and glucose in the blood; it also causes a disturbance of the pro-oxidant-antioxidant balance [9, 14].

Considering the complexity of the pathogenesis of cold injury, the premise of this scientific investigation is formed by results of the long-term research of the potential frigoprotectors with the various direction of action. On the model of acute hypothermia (cold air exposure) in mice numerous substances and medicines were studied, namely nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics-antipyretics (acetylsalicylic acid, ibuprofen, mefenamic acid, diclofenac sodium, meloxicam, celecoxib, etoricoxib, dual COX-2/5-LOX inhibitors – darbufelone, darbufelone mesylate, 5-(3,5-di-tert-butyl-4-hydroxybenzylidene)2-(thiazol-2-ylimino)-thiazolidin-4-one, COX-3 inhibitor paracetamol); CysLT1-leukotriene receptors blocker montelukast, recombinant interleukin-1 receptor antagonist ARIL-1; the drugs targeting metabolism through the different mechanisms – glucosamine hydrochloride, glucosamine sulphate, dietary supplement "Glucosamine-C BHFZ" the medicines containing quercetine – "Corvitin" (water-soluble drug), "Lipoflavon" (liposomal drug); liposomal drug "Lipin" containing phosphatidylcholine, ascorbic acid; actoprotector bemitil; adaptogens such as "Pollentar" containing pollen and succinic acid, aspen bark (Populus tremula L.) extract, and Rhodiola (Rhodiola rosea L.) liquid extract; intranasally administered oligopeptide drugs – homologs of the $ACTH_{15-18}$ fragment (under the codes KK-1 and KK-5) and "Semax" (in total, 27 medicines, dietary supplements, and compounds were investigated). NSAIDs and antileukotriene drugs have proved to be among the most effective frigoprotectors, and the leaders of screening have been identified such as diclofenac sodium at a dose of 14 mg/kg, etoricoxib at a dose of 10 mg/kg, darbufelone mesylate at a dose of 40 mg/kg, and montelukast at a dose of 2 mg/kg (the survival time of animals increased by 54 %, 38 %, 66 %, 45 % respectively) [15–17].

Thus, given the significant role of prostaglandins, thromboxane, leukotrienes, and other mediators in the pathogenesis of cold trauma [18–20], it is reasonable to conduct an in-depth study of arachidonic acid cascade inhibitors – NSAIDs – as promising frigoprotectors.

The **aim of the study** is to evaluate the effect of NSAIDs with various mechanisms of action on stress response and the functional state of the kidneys, liver, and heart on the model of acute general cooling.

2. Planning (methodology) of the research

Based on the results of previous studies [20–22], the drugs with different mechanisms of action were selected for an in-depth study of the effect of NSAIDs on the stress response and the functional state of the kidneys, liver, and heart in acute general cooling: diclofenac sodium is a non-selective COX inhibitor [23], etoricoxib is a highly selective COX-2 inhibitor [24], darbufelone mesylate is a dual COX-2/5-LOX inhibitor [25]. Each of these drugs is the leader of pre-screening among representatives of NSAIDs with different selectivity by the criterion of life expectancy increase on the model of AGC [20, 22].

The model of air hypothermia, an AGC, was chosen for the study [26].

Stages of the study:

1. Analysis of scientific data.

2. Modelling of a cold injury (AGC model) with control of body temperature dynamics.

3. Study of the excretory renal function in rats after AGC, including the groups under the use of the studied medicines.

4. Evaluation of the myocardium functional state by electrocardiogram (ECG) indicators.

5. Sampling of urine and blood serum, biochemical studies.

6. Processing and analysis of the obtained results.

7. Identification of promising areas for further research.

3. Materials and methods

The work was carried out in the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy during 2020–2021 in accordance with "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes" (Brussels, 2010) [27].

The protocols of the experiments were approved by the bioethics committee of the National Pharmaceutical University (Protocol No. 5, 25.03.2021). 35 adult random-bred male rats aged 8 months weighing 256±5 g were used. The animals were housed in standard polypropylene cages at a temperature of 20–26 °C and relative humidity of 50 % in properly ventilated rooms with a 12-hour day/night cycle and free access to food and water.

The following drugs and substances were used in the study: diclofenac sodium (Voltaren® tablets, Novartis, Switzerland), etoricoxib (Arcoxia®, tablets, Merck Sharp&Dohme Idea Inc, USA), darbufelone mesylate (synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry of the Danylo Halytsky Lviv National Medical University under the supervision of Professor R. B. Lesyk) [22].

Rats were randomly divided into 5 groups: the intact control group (n=7), the groups that underwent cold trauma exposure as follows: the untreated control group (n=7), the groups of animals that received intragastrically (i.g.) diclofenac sodium at a dose of 7 mg/kg (n=7), etoricoxib at a dose of 5 mg/kg (n=7), or darbufelone mesylate at a dose of 20 mg/kg (n=7) before cold trauma exposure.

The doses of diclofenac sodium, etoricoxib, and darbufelone mesylate were chosen based on the results of screening studies as the most effective in increasing the life expectancy of animals in acute hypothermia [20, 22].

The drugs and the substance of darbufelone mesylate were ground and suspended with the addition of

Tween-80, administered through an intragastric probe in a volume of 0.5 ml per 100 g of rat body weight 30 minutes before cold trauma exposure (preventive regimen) [26]. Animals of the intact control and the untreated control groups received i.e. drinking water in an equivalent volume.

AGC was used as a model of air hypothermia. The animals were placed in separate transparent plastic containers with a volume of 5000 cm3 without the restriction of motor activity and air access. Containers with rats underwent exposure at –18 °C for 2 hours in the freezer "Nord Inter-300." Body temperature was measured rectally for 5 minutes before and 5 minutes after cold exposure using a Microlife MT-1931 thermometer [26].

The excretory renal function (ERF) was evaluated 30 minutes after AGC. Water loading (3 % of body weight) was given to the animals i.e., they were placed in metabolic cages, and urine collection was carried out for 2 hours. 3 days before the experiment, the animals were previously adapted to the experimental conditions (gastric intubation, water load with the further staying in metabolic cages and urine collection procedures) [28].

3 hours after acute cold trauma, a 2-lead electrocardiogram (ECG) was recorded in rats under thiopental sodium anaesthesia (40 mg/kg intraperitoneally), at a tape speed of 50 mm/s using an EC1T-03M2 electrocardiograph [29]. The functional state of the heart was evaluated by the following ECG indicators: duration of the cardiac cycle – RR interval, duration of the PQ interval, which characterizes atrioventricular conduction, ventricular QRS complex duration, QT interval as a measure of ventricular electrical systole, as well as the voltage of the *P*, *T*, and *R* waves. The number of animals with ST-segment displacement relative to the isoline was determined, the heart rate (HR, beats per minute, BPM) and systolic index (SI) as the marker of contractile function were calculated [29].

Immediately after ECG recording, anesthetized rats were decapitated. Blood was collected and, after natural clot formation, serum was obtained. "Selye triad of stress" was chosen as a classic criterion of stress-protective action, and the relative weights of the thymus and adrenal glands, the number of gastric ulcers were registered in animals that underwent cold stress modelling. The thymus, adrenal glands, kidneys, and liver were taken and relative organ weights were calculated. The gastric mucosa was examined for erosions and ulcers [30].

In serum, photocolorimetric determination was performed for the values as follows: the activity of alanine aminotransferase (ALT) – according to the method of Reitman and Frankel, glucose – by the glucose oxidase method, cholesterol – by the enzymatic method, and total protein – by the reaction with the biuret reagent. The concentration of creatinine in urine and serum was determined by the Jaffe reaction [31]. Diuresis, ratio "excreted/administered fluid" GFR (using endogenous creatinine concentrations) were calculated.

Statistical processing of the results was done using the program "Statistica 10.0". The hypothesis of normality was rejected according to the Shapiro-Wilk test as well as asymmetry and excess coefficients values. The central tendencies of independent samples were compared using the Kruskal-Wallis test and the Mann-Whitney U test. The differences within the individual groups in the dynamics were assessed by the paired samples Wilcoxon test. Fisher's exact test was used to compare categorical variables. The differences were considered statistically significant at $p<0.05$. Quantitative data were presented as arithmetic means with standard errors of the mean ($M\pm m$), medians with 25 % and 75 % percentiles (Me [Q25; Q75]) and percentages.

4. Results

Cold exposure caused a statistically significant decrease in body temperature in rats of the untreated control group – by 1.8 °C on average ($p<0.05$). In animals receiving arachidonic acid cascade inhibitors, hypothermia was less pronounced, and the body temperature of animals after exposure at –18 °C did not significantly differ from the basal level (Table 1).

Diclofenac sodium was the most effective in the prevention of hypothermia, as evidenced by the statistically significant differences ($p<0.05$) compared with the untreated group values. The effect of etoricoxib and darbufelone mesylate on the severity of hypothermia was observed only as a tendency towards the increase in body temperature.

Table 1

Body temperature changes (measured rectally) in rats after acute general cooling under administration of the arachidonic acid cascade inhibitors, $M\pm m$; Me [Q25; Q75]

Group	Body temperature, °C		
	Basal level	After cold influence	Difference
Intact control, n=7	37.5±0.2 37.4[37.0;38.0]	–	–
Untreated control (AGC), n=7	38.0±0.3 38.0[37.6;38.6]	36.2±0.9* 37.4[34.0;37.5]*	–1.8±0.8 –1.3[–2.5;–0.4]
Diclofenac sodium+AGC, n=7	38.0±0.2 38.0[37.8;38.3]	38.1±0.1 38.0[37.6;38.3]	0.0±0.2# –0.2[–0.3;0.4]#
Etoricoxib+AGC, n=7	38.0±0.3 38.1[37.4;38.3]	37.5±0.5 37.6[37.1;38.5]	–0.5±0.4 0.1[–1.6;0.4]
Darbufelone mesylate+AGC, n=7	37.6±0.3 37.5[37.2;38.0]	36.8±0.5 37.4[35.8;37.5]	–0.8±0.5 –0.9[–1.4;–0.1]

*Note: AGC – acute general cooling. Statistically significant differences: compared to basal level values – * ($p<0.05$), compared to the untreated control group values – # ($p<0.05$)*

The stress-protective activity of arachidonic acid cascade inhibitors under AGC was evaluated by the Selye triad. The results of the study are shown in Table 2.

The Selye triad observed in the untreated animals was characterized by a statistically significant increase in the relative adrenal glands weight as well as in the frequency of ulceration, and a reduction in the relative thymus weight ($p<0.05$) compared to the intact control group values. The obtained results indicate the development of marked stress response under acute cold trauma.

Table 2

Stress-protective effect of the arachidonic acid cascade inhibitors under acute general cooling, *M*±*m*; Me [Q25; Q75]

Group	Relative adrenal glands weight	Relative thymus weight	The stomach	
			Incidence of gastric ulcers, %/ absolute quantity	Number of ulcers per group/per 1 animal
Intact control, *n*=7	0.023±0.001 0.023 [0.022;0.024]	0.059±0.007 0.059 [0.039;0.072]	0 0/7	0/0
Untreated control (AGC), *n*=7	0.031±0.002* 0.028 [0.026;0.035]*	0.038±0.005* 0.044 [0.025;0.048]*	57.1* 4/7*	23/3.29
Diclofenac sodium+AGC, *n*=7	0.025±0.001# 0.025 [0.023;0.026]#	0.060±0.005## 0.055 [0.051;0.072]##	85.7** 6/7**	23/3.29
Etoricoxib+AGC, *n*=7	0.024±0.001 0.024 [0.020;0.028]	0.058±0.005# 0.061 [0.050;0.069]#	28.6&& 2/7&&	2/0.29
Darbufelone mesylate+AGC, *n*=7	0.023±0.002# 0.022 [0.019;0.027]#	0.065±0.006## 0.070 [0.051;0.081]##	42.9& 3/7&	12/1.71

*Note: AGC – acute general cooling. Statistically significant differences: compared to the intact control group values – * (p<0.05), ** (p <0.01); compared to the untreated control group values – # (p<0.05), ## (p<0.01); compared to diclofenac sodium group values – & (p<0.05), && (p<0.01)*

Diclofenac sodium and darbufelone mesylate prevented the development of adrenal hypertrophy and thymus involution, since the normalization of the relative weights of these organs was observed in comparison with the indicators of the untreated control group ($p<0.05$ and $p<0.01$, respectively in both groups, Table 2). Similar changes, albeit less pronounced, were seen in animals receiving etoricoxib.

The incidence of gastric ulcers after the use of arachidonic acid cascade inhibitors did not significantly differ from the value of the untreated control group. At the same time, there was a tendency towards the decrease in this value in groups receiving the selective COX-2 inhibitors – darbufelone mesylate and etoricoxib, and in these groups there were no statistically significant differences with the intact control group values. Administration of diclofenac sodium, which is a non-selective COX inhibitor, was accompanied with a significantly higher incidence of gastric ulcers compared to the intact control group (p<0.01), still the number of ulcers per group as well as per 1 animal did not exceed the values of the untreated control group. According to the number of ulcers formed per 1 animal, the groups were arranged in the following order: untreated control (AGC)=diclofenac sodium+AGC group>darbufelone mesylate+AGC group>etoricoxib+AGC group.

The effect of AGC and its course under the influence of arachidonic acid cascade inhibitors on the ERF was evaluated under conditions of water loading induced diuresis 30 minutes after cold exposure (Tables 3, 4).

AGC caused the ERF impairment. In the untreated control group, GFR equalled 56 % of the intact animals value ($p>0.05$). Diuresis and ratio "excreted/ administered fluid" were significantly reduced by more than 2.5 times ($p<0.01$). All the studied drugs significantly improved these indicators, although they did not restore them to the level of intact animals. The influence on ERF is probably associated with a less marked decrease in GFR in rats under ACG after NSAID administration. There were no statistically significant intergroup differences in water reabsorption values.

Proteinuria as well as protein excretion did not undergo significant changes compared to intact control values either in the group of untreated control or in the groups receiving the studied drugs. Only after etoricoxib administration the statistically significant decrease in proteinuria was seen, relative to that in the group of untreated animals (Table 4).

Table 3

The changes in the excretory renal function in rats after acute general cooling under the administration of the arachidonic acid cascade inhibitors, *M*±*m*; Me [Q25; Q75]

Group	Values			
	Diuresis, ml/100 g for 2 h	Ratio "excreted/ administered fluid," %	Glomerular filtration rate, ml/ min for 100 g	Water reabsorption, %
Intact control, *n*=7	2.13±0.06 2.15 [1.94;2.24]	71.0±1.9 71.6 [64.5;74.2]	0.39±0.07 0.37 [0.27;0.53]	94.39±1.25 95.53 [94.02;96.63]
Untreated control (AGC), *n*=7	0.80±0.12** 0.83 [0.53;1.06]**	26.7±4.1** 27.6 [17.5;35.1]**	0.22±0.05 0.18 [0.16;0.27]	96.73±0.38 97.19 [95.66;97.44]
Diclofenac sodium+AGC, *n*=7	1.22±0.13**#$ 1.22 [1.07;1.40]**#$	40.8±4.2**#$ 40.7 [35.8;46.7]**#$	0.29±0.03 0.28 [0.25;0.36]	96.48±0.31 96.38 [96.21;96.89]
Etoricoxib+AGC, *n*=7	1.36±0.10**##$ 1.35 [1.21;1.53]**##$	45.1±3.2**#$ 44.6 [40.2;50.6]**#$	0.32±0.03 0.35 [0.23;0.38]	96.32±0.31 96.75 [95.70;97.01]
Darbufelone mesylate+AGC, *n*=7	1.67±0.11**##&^ 1.57 [1.46;1.83]**##&^	56.6±3.6**##&^ 56.0 [48.7;61.2]**##&^	0.34±0.02## 0.34 [0.30;0.36]##	95.85±0.31 95.78 [95.33;96.43]

*Note: AGC – acute general cooling. Statistically significant differences: compared to the intact control group values – ** (p<0.01); compared to the untreated control group values – # (p<0.05), ## (p<0.01); compared to diclofenac sodium group values – & (p<0.05); compared to darbufelone mesylate group values – $ (p<0.05)*

The relative kidneys weight in the group receiving diclofenac sodium was slightly reduced compared to the intact control value (p<0.05). In other cases, no significant intergroup differences were observed.

The effect of NSAIDs on the heart functional state under AGC was evaluated by the changes in ECG parameters (Table 5).

The experimental AGC was characterized by specific changes in ECG parameters (Table 5). In animals of the untreated control group, on the background of sinus rhythm, a significant prolongation of the QT interval by 23.4 % compared to intact control rats value (p<0.05) was observed, indicating a prolongation of ventricular electrical systole and being a predictor of severe cardiac arrhythmias [32]. A decrease in myocardial contractile function was also seen, which was confirmed by a statistically significant increase in the systolic index by 25 %. The trophic processes in the myocardium underwent disturbance which was indicated by a shift of the ST segment relative to the isoline [32] in 2 out of 7 (28.6 %) animals of the untreated control group (p>0.05 compared to the intact control data). AGC did not affect atrioventricular conduction, as evidenced by the absence of significant changes in the PQ interval compared to the same indicator in the intact control group. A tendency towards the reduction of the QRS complex duration indicates an acceleration of intraventricular impulse conduction under cold trauma [32]. No significant changes in the voltage of the *P*, *R*, and *T* waves were observed.

All studied NSAIDs prevented the negative effect of AGC on the cardiac contractile function, which was confirmed by a statistically significant decrease in the systolic index (p<0.05 compared to the untreated control data), which approximated to the values of the intact animals. In addition, the drugs contributed to the normalization of the QT interval thus indicating a decrease in the risk of arrhythmia. However, etoricoxib and darbufelone mesylate enhanced AGC influence on intraventricular conduction. This is evidenced by a statistically significant decrease in the duration of the QRS complex compared to the intact control and the untreated control data. Diclofenac sodium did not enhance the effect of AGC on ventricular depolarization (p<0.05 compared to the intact control group, p>0.05 compared to the untreated control group).

A 21.2 % reduction in the PQ interval indicates an acceleration of atrioventricular conduction on the background of diclofenac sodium (p<0.05 compared to the intact control data). A similar tendency was observed after administration of the selective COX-2 inhibitor etoricoxib (reduction by 10.9 %, p>0.05). Darbufelone mesylate did not exert any significant effect on the PQ interval.

Prevention of the disorders of trophic processes in the myocardium is evidenced by the absence of animals with ST-segment displacement after diclofenac sodium and etoricoxib administration, but not in the group receiving darbufelone mesylate. In the latter, the absolute and relative number of rats with ST-segment displacement did not differ from the respective values of the untreated control group.

No significant intergroup differences were observed regarding the voltage of the P, R, and T waves, neither the intergroup differences heart rate reached statistical significance because of high dispersion. There was a tendency towards the increase in heart rate after diclofenac sodium administration compared to other groups.

The effect of arachidonic acid cascade inhibitors on liver function after cold exposure was evaluated by ALT activity, total protein, glucose, and cholesterol serum concentrations (Table 6).

Serum ALT activity did not show any intergroup differences, and only in the untreated control group there was a tendency towards its elevation. Serum glucose and cholesterol levels also did not undergo significant changes in any of the groups relative to the intact control values. Notably, a mild increase in the concentration of glucose was observed in serum of animals in all groups, including the intact control. This can be explained by the effect of thiopental sodium anaesthesia [33].

The total protein concentration increased significantly in animals of the untreated control group, while in rats receiving etoricoxib and darbufelone mesylate, there was a tendency towards its elevation. Diclofenac sodium contributed to the normalization of total protein concentration in serum (p>0.05 compared to the intact control group, p<0.05 compared to the untreated control group) under AGC. It should be noted that the detected fluctuations in this value are within the physiologically normal ranges in the untreated control group as well as in the group receiving diclofenac sodium.

Table 4

Relative kidneys weight, protein concentration in urine and its excretion in rats after acute general cooling under the arachidonic acid cascade inhibitors administration, *M*±*m*; Me [Q25; Q75]

Group	Value		
	Proteinuria, g/l	Protein excretion, mg/100 g for 2 h	Relative kidneys weight, %
Intact control, *n*=7	0.08±0.03 0.05[0.02;0.09]	0.17±0.07 0.10[0.06;0.20]	0.70±0.02 0.70[0.63;0.76]
Untreated control (AGC), *n*=7	0.12±0.03 0.08[0.05;0.18]	0.09±0.02 0.10[0.03;0.15]	0.67±0.05 0.61[0.59;0.71]
Diclofenac sodium+AGC, *n*=7	0.11±0.06 0.06[0.04;0.07]	0.10±0.03 0.08[0.06;0.08]	0.60±0.03* 0.59[0.57;0.62]*
Etoricoxib+AGC, *n*=7	0.04±0.01# 0.04[0.03;0.07]#	0.06±0.01 0.05[0.04;0.09]	0.65±0.02 0.65[0.62;0.71]
Darbufelone mesylate+AGC, *n*=7	0.05±0.01 0.05[0.01;0.06]	0.08±0.02 0.09[0.03;0.11]	0.64±0.04 0.66[0.63;0.70]

*Note: AGC – acute general cooling. Statistically significant differences: compared to the intact control group values – * (p <0.05); compared to the untreated control group values – # (p<0.05)*

Table 5

Electrocardiographic parameters of the functional state of the heart of rats after acute general cooling under the arachidonic acid cascade inhibitors administration, *M*±*m*; Me [Q25; Q75]

Values	Intact control	AGC			
		Untreated control (AGC), *n*=7	Diclofenac sodium+AGC, *n*=7	Etoricoxib+AGC, *n*=7	Darbufelone mesylate+AGC, *n*=7
PQ, s	0.046±0.009 0.050 [0.040;0.050]	0.047±0.005 0.050 [0.040;0.050]	0.036±0.004*####$$$ 0.035 [0.035;0.040] *####$$$	0.041±0.002#$$ 0.040 [0.040;0.040] #$$	0.048±0.004&&&^^ 0.050 [0.045;0.050] &&&^^
QRS, s	0.037±0.011 0.030 [0.030;0.050]	0.029±0.006 0.30 [0.025;0.030]	0.024±0.012* 0.020 [0.020;0.020] *	0.020±0.000****### 0.020 [0.020;0.020] ****###	0.019±0.002****### 0.020 [0.020;0.020] ****###
QT, s	0.064±0.010 0.060 [0.055;0.070]	0.079±0.004* 0.080 [0.080;0.080] *	0.058±0.008####$ 0.060 [0.060;0.060] ####$	0.066±0.005### 0.070 [0.060;0.070] ###	0.065±0.004###& 0.065 [0.060;0.070] ###&
R, mV	0.80±0.30 0.75 [0.75;1.13]	0.80±0.39 0.89 [0.44;1.13]	0.75±0.14 0.69 [0.69;0.89]	0.55±0.21 0.56 [0.38;0.75]	0.80±0.22 0.75 [0.63;1.00]
P, mV	0.12±0.08 0.13 [0.09;0.13]	0.14±0.04 0.016 [0.013;0.19]	0.15±0.05 0.16 [0.09;0.19]	0.12±0.02 0.13 [0.13;0.13]	0.13±0.00 0.13 [0.13;0.13]
T, mV	0.19±0.08 0.19 [0.13;0.22]	0.17±0.07 0.19 [0.09;0.22]	0.16±0.07 0.19 [0.06;0.22]	0.17±0.04 0.19 [0.13;0.19]	0.18±0.04 0.19 [0.16;0.19]
HR, beats per minute	410.2±67.4 428.6 [400.0;461.5]	412.8±21.4 428.6 [400.0;428.6]	448.8±32.4 461.5 [428.6;461.5]	421.0±37.7 428.6 [400.0;461.5]	425.7±27.6 428.6 [400.0;461.5]
SI, %	43.2±9.4 42.9 [36.7;46.7]	54.0±3.2* 53.3 [50.0;57.1] *	43.0±4.5#### 43.3 [42.9;46.2] ####	46.0±4.7# 46.2 [41.4;50.0] #	46.1±3.2### 46.7 [42.9;50.0] ###
Animals with ST-segment displacement, %/absolute quantity)	0 % 0/0	28.6 % 2/7	0 % 0/0	0 % 0/0	28.6 % 2/7

*Note: AGC – acute general cooling, HR – heart rate, SI – systolic index. Statistically significant differences: compared to the intact control group values – * (p<0.05), **** (p<0.001); compared to the untreated control group values – # (p<0.05), ### (p<0.005), #### (p<0.001); compared to etoricoxib group values – ^^ (p<0.01); compared with darbufelone mesylate group values – $ (p <0.05), $$ (<0.01), $$$ (p<0.005)*

Table 6

Biochemical markers of the functional state of the liver in rats after acute general cooling under the arachidonic acid cascade inhibitors administration, *M*±*m*; Me [Q25; Q75]

Group	Value			
	ALT activity, μmol/(h×ml)	Glycemia, mmol/l	Cholesterol concentration, mmol/l	Total protein, g/l
Intact control, *n*=7	0.94±0.24 0.95 [0.53;1.34]	8.53±0.35 8.69 [8.02;9.04]	1.37±0.06 1.38 [1.27;1.47]	61.9±1.2 61.9 [59.1;64.4]
Untreated control (AGC), *n*=7	1.03±0.14 1.08[0.85;1.13]	7.04±0.41 7.26 [6.19;7.59]	1.45±0.29 1.68 [1.22;1.77]	68.2±1.5* 69.2 [65.2;71.7]*
Diclofenac sodium+AGC, *n*=7	0.65±0.04 0.66 [0.59;0.70]	7.10±0.47 7.33 [6.50;7.70]	1.48±0.09 1.49 [1.33;1.62]	62.5±1.4# 61.9 [61.1;65.6]#
Etoricoxib+AGC, *n*=7	0.84±0.32 0.71 [0.34;1.34]	7.56±0.40 7.69 [6.89;8.22]	1.19±0.06 1.22 [1.11;1.27]	65.1±3.0 64.4 [61.1;66.8]
Darbufelone mesylate+AGC, *n*=7	0.90±0.27 0.72 [0.49;0.88]	7.25±0.68 7.09 [6.19;7.78]	1.60±0.11^ 1.57 [1.38;1.72]^	65.9±2.2 66.0 [58.7;69.6]

*Note: AGC – acute general cooling. Statistically significant differences: compared to the intact control group values – * (p<0.05); compared to the untreated control group values – # (p<0.05); compared to etoricoxib group values – ^ (p<0.05)*

Turning to the relative liver weight, which equalled 3.2±0.1 %; 3.2[3.1;3.4] in the intact control group, a mild decrease was recorded in the untreated control group (2.8±0.1 %; 2,8[2.6;3.0], *p*<0.05) and on the background

of diclofenac sodium (2.9±0.1 %; 2.8[2.5;3.0], $p<0.05$). Both in the etoricoxib group and darbufelone mesylate group no significant changes were registered, and the values equalled 3.0±0.2 %; 3.0[2.5;3.4] and 3.0±0.2 %; 3.2[2.7;3.4] respectively.

5. Discussion

As shown in Table 1, all the studied drugs decreased the severity of hypothermia. These results correspond to the previously obtained data on the frigoprotective properties of arachidonic acid cascade inhibitors [20–22]. Diclofenac sodium is particularly distinguished by its ability for complete prevention of a decrease in rectally measured body temperature in rats under AGC.

The stress-protective activity of diclofenac sodium, etoricoxib, and darbufelone mesylate under cold trauma is evidenced by the criteria of "Selye triad of stress" namely the relative weights of the thymus and adrenal glands, the number of gastric ulcers (Table 2). All the studied drugs contribute to the normalization of the relative weights of the aforementioned organs.

Etoricoxib, being a highly selective COX-2 inhibitor with barely any effect on prostaglandin E2 synthesis, is thus reasonably characterized by the absence of ulcerogenic action, which was indicated by lower incidence of gastric ulcers compared to diclofenac sodium group. A similar tendency is observed for the dual COX-2/5-LOX inhibitor darbufelone mesylate. A tendency towards the increase in the incidence of gastric ulcers on the background of diclofenac sodium compared to the untreated control group is obviously associated with the gastrotoxic effect typical of non-selective COX inhibitors [34]. It should be emphasized that despite the ulcerogenic potential, diclofenac sodium did not potentiate stress-induced gastrotoxicity during AGC, which is evidenced by the absence of the statistically significant differences in the incidence of gastric ulcers compared to the untreated control group.

The stress-protective effect of NSAIDs may be related to their influence on the prostaglandins levels, which are known to modulate the secretory activity of the hypothalamic-pituitary-adrenal axis [35]. Acetylsalicylic acid, a non–selective COX inhibitor, was shown to inhibit the stress-related secretion of adrenocorticotropic hormone and cortisol [36]. However, the role of arachidonic acid cascade inhibitors in modulating the activity of this hormonal axis at rest and under stress-related conditions, especially before, during, and after cold exposure, requires further studies.

Notably, the renal effects of NSAIDs are being changed qualitatively under acute exposure to cold, namely the nephrotoxic effect (typical of these drugs under normal conditions) is not manifested. Acute kidney injury induced by NSAIDs is primarily associated with the effect on renal hemodynamics and is accompanied with the disorders of the fluid/electrolyte balance (sodium retention, water retention, increased blood pressure, edema). Moreover, the development of the acute interstitial nephritis is possible, and it can be manifested by nephrotic proteinuria [37]. In contrast, under AGC, the studied NSAIDs reduced the severity of ERF impairment. According to the positive influence on the kidneys function the studied drugs can be arranged in the following order: diclofenac sodium=etoricoxib<darbufelone mesylate (Tables 3, 4).

It is well known that the risk of ulcerogenic effects and impairement of renal function correlates with the selectivity of certain NSAIDs for COX-1/COX-2. It is attributable to the important role of prostaglandins in protecting the gastric mucosa and maintaining renal circulation [34, 37]. The absence of an increase in diclofenac sodium gastrotoxicity as well as and nephrotoxicity is presumably caused by the reduction of the degree of COX inhibition in cold exposure. This is confirmed by the results of a study [38] which addressed the dependence of diclofenac sodium anti-inflammatory effect on the temperature regimen and shown that this effect is practically not observed under hypothermia.

Considering the fact that the cold factor leads to disorders of the autonomic innervation, rhythm, and conduction of the heart [1, 4], the effect of prospective frigoprotectors on the cardiovascular system was studied under AGC (Table 5). An increase in the systolic index and prolongation of the QT interval in the untreated control group indicates an impairment of myocardial contractile function and is also a marker of arrhythmogenic changes caused by cold exposure [32].

The similarity of the heart rate values in the experimental groups enabled to estimate the physiological meaning of systolic index normalization in animals receiving arachidonic acid cascade inhibitors under AGC: it indicates an enhancement in the myocardial contractile function [39]. Normalization of the QT interval after the use of diclofenac sodium, etoricoxib, darbufelone mesylate is a favourable predictor indicating a reduced risk of cardiac arrhythmias compared to the untreated control group data. Diclofenac sodium, in contrast to etoricoxib and darbufelon mesylate, does not increase the negative effect of cold trauma on the ventricular depolarization.

The absence of the significant changes in the functional state of the liver (Table 6) may result from the relatively short-term cold exposure and early term of the biochemical studies. Thus, in further studies it is reasonable to address the dynamics of biochemical markers of the liver function in the delayed period after acute cold trauma, as well as under chronic exposure to low temperatures.

Considering the above, it could be assumed that the frigoprotective effect of NSAIDs could be associated not only with the influence on the synthesis of inflammatory mediators but with the other specific mechanisms that require further studies.

The obtained results experimentally substantiate the possibility of arachidonic acid cascade inhibitors prophylactic use to reduce the severity of stress response and impairments of the functional state of the kidneys and heart under cold trauma.

Study limitation: The mechanisms of frigoprotective activity of NSAIDs at the level of their influence on various pathways of the arachidonic acid cascade in normo- and hypothermia were not determined in this study.

Further research prospects: Elucidation of the effect of acute general cooling on the activities of enzymes of the arachidonic acid cascade and on metabolites (thromboxane, prostaglandins, leukotrienes) concentrations. Studies addressing the molecular mechanisms of frigoprotective activity of NSAIDs with various mechanisms of action.

6. Conclusions

1. Diclofenac sodium, etoricoxib, and darbufelone mesylate, when administered in preventive regimen, demonstrate frigoprotective properties in a model of acute general cooling in the rat caused by exposure at –18 °C for 2 hours. The non-selective COX inhibitor diclofenac sodium is the most effective in preventing a decrease in body temperature.

2. The stress-protective effect of the studied arachidonic acid cascade inhibitors is manifested in the prevention of adrenal hypertrophy and thymus involution, and the beneficial effect on the kidneys is indicated by the reduction of the severity of the excretory renal function impairment and maintenance the ability to excrete water loading.

3. All studied NSAIDs contribute to ECG normalization. They prevent a decrease in the myocardial contractile function (proceeding from the systolic index) as well as prolongation of the QT interval caused by acute cold trauma. Diclofenac sodium, in contrast to etoricoxib and darbufelone mesylate, does not increase the effect of cold on ventricular depolarization.

4. The studied NSAIDs do not cause any negative changes in the main markers of the liver function under acute general cooling model.

Conflict of interest

The authors declare that they have no conflicts of interest.

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