The study of the effect of preparations of glucosamine and acetylsalicylic acid on the behavioral reactions and the physical endurance of rats on the model of acute local cold trauma

Aim. To study the effect of preparations of glucosamine and acetylsalicylic acid on behavioral actions and physical endurance on the model of acute local cold trauma (CT) in rats.

Materials and methods. Contact frostbite was modeled by V. V. Boyko method. The rectal temperature was determined in 40 min after the simulation of the local CT. The state of the central nervous system was assessed 1 hour after CT by behavioral reactions in the "open field" test. The motor activity, orienting research activity, emotional reactions and their vegetative support, number of fecal boluses and urinations were determined.

Results and discussion. It has been found that the drugs studied assist to normalization of the rectal temperature, as well as improve the dynamics of recovery of behavioral reactions and the physical activity in rats after acute local cold trauma.

Conclusions. On the model of forced swimming with a load after acute local trauma acetylsalicylic acid increased the time of the physical fatigue onset by 1.4 times, Glucosamine-C BCPP by 1.7 times and glucosamine hydrochloride by 1.66 times. Preparations of glucosamine showed the best protective effect.

Key words: local cold trauma; behavioral reactions; physical endurance; acetylsalicylic acid; glucosamine
Introduction. In the modern world people are affected by a large number of negative environmental factors. Emergency work, adverse climatic conditions, psychotechnogenic impacts, and combat actions are major stress-related factors [1-4]. The damage of the body with low environmental temperatures is very important. Cold traumas (CT) in the structure of injuries in peacetime are 3-10 % and accompanied by high disability of victims and mortality [5, 6].

The pathogenesis of the low body temperature affects changes in the central nervous, cardiovascular, endocrine, immune, respiratory, excretory systems, gastrointestinal tract and skin [7].

The arsenal of medicines with the frigoprotective action is rather narrow. In addition, most of these agents affect only one or more components of the pathogenesis of CT. Therefore, the search for new safe and effective frigoprotectors is important. Convincing evidence of the effectiveness of glucosamine hydrochloride (G h/ch) in CT was obtained. G h/ch and the dietary supplement Glucosamine-C BCPP containing G h/ch and ascorbic acid in acute CT has a distinct protective effect: reduces hypothermia, improves animal survival, the motor and physical activity, normalizes rheological properties of the blood, exceeding acetylsalicylic acid (ASA) – the known frigoprotector [8-10]. Under the conditions of CT ASA, Glucosamine-C BCPP and especially G h/ch exhibit distinct anti-inflammatory properties as evidenced by a significant decrease in interleukin IL-1b and an increase in the content of IL-10 [11]. On the acute CT model the highest frigoprotective effect was shown by G h/ch and Glucosamine-C BCPP when no inflammatory reactions and microcirculation disorders in the skin were observed; under the effect of G h/ch the histological structure of the skin was close to that in the intact control group [12].

After 14 days of taking the dietary supplement Glucosamine-C BCPP under the effect of cold during field exercises the cadets of the National Academy of the National Guard of Ukraine had a reduced risk of acute respiratory diseases, an increased adaptation of the organism to a long-lasting effect of low temperatures, an increased concentration of attention and mental working capacity [13].

G h/ch is an effective antihypothermic and antioxidant agent in the local CT [14]. The nootropic action of G h/ch [15] was revealed. This suggests the ability of G h/ch to improve behavioral reactions and physical endurance in CT. The clarification of this ability was the purpose of this study.

The aim of the work was to determine the effect of preparations of glucosamine and acetylsalicylic acid on the body temperature, behavioral responses and physical endurance of rats in conditions of acute local CT.

Materials and methods. Experiments were done on 30 white outbred male rats weighing 200-250 g. Animals were kept on a standard diet of the vivarium with free access to water, with constant temperature and humidity. The protocol of the study is consistent with bioethical standards and corresponds to the “General ethical principles of animal experimentation” (Ukraine, 2001) and is not in contradiction with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) [16].

Contact frostbite was modeled by V. V. Boyko method [17] under thiopental anesthesia (40 mg/kg) on the depilated area of the back skin 1.5 cm away from the spine. A copper plate of 3.5 × 3.0 cm pre-cooled in liquid nitrogen (-196 °C) was applied to the depilated skin for 3 min. This method allows obtaining cold skin lesions that are standard by area and depth and correspond to the III degree of frostbite by clinical classification. The rectal temperature was determined by a WSD-10 digital thermometer in 40 min after the simulation of the local CT.

The state of the central nervous system (CNS) was assessed 1 hour after CT by behavioral reactions in the “open field” test [18]. The motor activity, orienting research activity, emotional reactions and their vegetative support, number of fecal boluses and urinations were determined [16]. Physical endurance of animals was studied 20-30 min after the “open field” test on the model of forced swimming with a load (7.5 % of the body weight at the root of the tail). The criterion for the total exhaustion was the 10-second stay of animals under water and the inability to come to the surface to breathe [5].

The following drugs and substances were used in the study: 0.9 % NaCl solution, Glucosamine-C BCPP (PJSC “Borschagovsky CPP”, Ukraine), glucosamine hydrochloride (G h/ch) substance (Sigma-Aldrich, Germany) and soluble ASA tablets (Bayer, Germany). They were administered as a water solution intragastrically (i/g) in the prophylactic mode 30 min before CT. Animals were divided into 5 groups: group 1 – intact control (IC) (n = 6), rats were injected NaCl (1 ml/100 g); group 2 – control pathology (CT) (n = 6), rats were injected NaCl (1 ml/100 g); group 3 – ASA (25 mg/kg) + CT (n = 6); group 4 – Glucosamine-C BCPP in the dose of 82.5 mg/kg corresponding to 50 mg/kg of G h/ch, + CT (n = 6); group 5 – G h/ch (50 mg/kg) + CT (n = 6). Doses of G h/ch of 50 mg/kg and ASA of 25 mg/kg are conventionally effective in CT [19].

The statistical processing of the results was carried out by the methods of variation statistics using “Statistics, V. 6.0” statistical program standard package with the calculation of the average value and its standard error, the significance of differences by Student’s criterion (t) for the normal distribution. In its absence the non-parametric White’s test was used. Differences were considered statistically significant if p < 0.05 [20].

Results and discussion. One hour after the end of the simulation of acute local CT a distinct hypothermia and a severe general condition of animals were observed (Tab. 1).

In all experimental groups a significant reduction in the rectal temperature (p < 0.05) was observed compared to the intact control group. Thus, in the group of CT it decreased on average by 1.8 °C, in the ASA group – by 1.58 °C, in the group of Glucosamine-C BCPP – by 1.4 °C and in the G h/ch group – by 1.22 °C. Under the effect of Glucosamine-C BCPP and G h/ch a tendency to re-
store the parameters of the rectal temperature compared to the group of intact animals. G h/ch had the best effect on temperature: the temperature was significantly higher (p < 0.05) compared to the ASA group.

The effect of acute local CT on the state of the CNS of rats after 3 days was characterized by a change in the parameters of behavioral reactions in the “open field” test (Tab. 2).

In the CT group there was a significant decrease in the locomotor, orienting research activity, vegetative support and emotional reactions (number of boluses and grooming acts) of rats, as well as the total activities compared to the intact control. This kind of change may indicate a significant inhibition of the CNS on the background of hypothermia and endotoxicosis as a result of severe local CT.

After the administration of ASA, Glucosamine-C BCPP, and G h/ch these indicators were significantly lower than those in the intact control group, but there was a significant increase in the locomotor activity on the background of all drugs and the total activities under the effect of all drugs compared to the indicators of the CT group. It indicates an increase in the tone of the CNS. By a positive effect on the orienting research activity G h/ch significantly exceeded than ASA.

On the background of acute local CT the physical endurance of rats decreased by 1.7 times by the time of forced swimming with a load (Tab. 3). ASA, Glucosamine-C BCPP and G h/ch caused a positive effect on this indicator in relation to the CT group. ASA increased the time of fatigue by 1.4 times, Glucosamine-C BCPP – by 1.7 times, G h/ch – by 1.66 times. Taking into account the positive effect on this indicator Glucosamine-C BCPP and G h/ch reveal marked actoprotective properties.

Thus, the results of the study of the effect of glucosamine preparations on the rectal temperature, behavioral responses in the “open field” test and physical endurance in acute local CT indicate the positive effect of

### Table 1

<table>
<thead>
<tr>
<th>Period of observation</th>
<th>Animal groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact control</td>
</tr>
<tr>
<td>Before cold trauma</td>
<td>39.20 ± 0.07</td>
</tr>
<tr>
<td>30 min after cold trauma</td>
<td>–</td>
</tr>
</tbody>
</table>

Notes: * – significant in relation to the intact control, p < 0.05; ** – significant in relation to G h/ch, p < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Markers (for 3 min)</th>
<th>Intact control</th>
<th>Acute local cold trauma (stress)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control pathology CT</td>
</tr>
<tr>
<td>Locomotor activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passed squares</td>
<td>52.83 ± 7.85</td>
<td>5.0 ± 1.24*</td>
</tr>
<tr>
<td>Holes</td>
<td>16.67 ± 2.95</td>
<td>2.67 ± 0.96*/***</td>
</tr>
<tr>
<td>Postures</td>
<td>10.83 ± 1.94</td>
<td>1.33 ± 0.62*/***</td>
</tr>
<tr>
<td>Fecal boluses</td>
<td>1.83 ± 0.48</td>
<td>0.33 ± 0.21*</td>
</tr>
<tr>
<td>Urinations</td>
<td>0.33 ± 0.21</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Grooming</td>
<td>0.83 ± 0.65</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>The total activities</td>
<td>83.33 ± 11.54</td>
<td>9.33 ± 1.61*/***</td>
</tr>
</tbody>
</table>

Notes: * – significant in relation to the intact control, p < 0.05; ** – significant in relation to the control pathology; *** – significant in relation to the parameters of the G h/ch group, p < 0.05.
these agents on the CNS, the functional state of the skeletal muscles, and prove their frigoroprotective properties.

Restoration of the rectal temperature prevents severe disorders of the CNS. In acute local CT preparations of glucosamine have marked actoprotective properties. The results of the previous studies [21] on the positive effect of G h/ch on the parameters of the motor and research activity, the muscle tone, coordination of movements, and physical endurance of mice in acute general CT confirmed this study. Thus, the conclusion can be made that G h/ch has a marked frigoroprotective effect for different types of CT (general and local).

**Conclusions and prospects for further research**

1. Preparations of glucosamine – Glucosamine-C BCPP and glucosamine hydrochloride – in acute local cold trauma (frostbite) prevent the decrease of the body temperature in rats, while glucosamine hydrochloride significantly exceeds the effect of acetylsalicylic acid.

2. Acetylsalicylic acid, Glucosamine-C BCPP, and especially glucosamine hydrochloride, assist to normalization of behavioral reactions in rats after acute local cold trauma according to the results of the “open field” test.

3. On the model of forced swimming with a load glucosamine preparations significantly increase the physical endurance of rats compared to the group of cold trauma, while glucosamine hydrochloride by its actoprotective activity is significantly more effective than acetylsalicylic acid.

4. The results of experiments indicate that the use of glucosamine preparations as frigoroprotectors in acute local cold trauma is promising.

**Conflict of interests:** authors have no conflict of interests to declare.

**REFERENCES**


REFERENCES


