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ACUTE HEAT TRAUMA MODEL IN RATS, GENDER-DEPENDENT THERMORESISTANCE, AND SCREENING OF POTENTIAL THERMOPROTECTORS

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Heat trauma (HT) is an urgent medical and social problem. Heat damage is a widespread effect of the environment on humans, driven by global warming, military conflicts, technological disasters, work in hot environments, and engagement in extreme sports and tourism.

The aim of the study: *to propose a model of acute HT in rats that does not cause the death of animals, to determine the dependence of thermoresistance on gender, and to compare the effectiveness of the thermoprotective effects of a range of non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and glucosamine hydrochloride in this model.*

Materials and methods: *The experiment was conducted on adult white rats of both genders. Acute HT was modelled by using a specially developed method involving heat exposure to animals at +55 °C for 30 minutes, followed by a recovery period of 60 minutes. Rectal temperature was measured every 15 minutes. The degree of hyperthermia in males and females was determined. The presence and intensity of the thermoprotective effect of glucosamine hydrochloride (G h/ch), diclofenac sodium, acetylsalicylic acid (ASA), nimesulide, etoricoxib, celecoxib, and paracetamol were evaluated through intragastric administration 60 minutes before heat exposure. The results were analyzed using the STATISTICA 12.0 program.*

Results: *It was established that heat exposure at +55 °C for 30 minutes effectively replicates acute HT in rats without causing animal fatalities, adhering to bioethical requirements. Body temperature increases by 10–13 %, characterized as a heat stroke. Occasionally, thermoresistant animals are encountered, where the temperature increase during the first 15 minutes of exposure is less than 1 °C. These animals should not be used for further modelling of heat trauma. Male rats are more sensitive to the effect of high environmental temperatures than females, exhibiting greater hyperthermia (temperature increase of 5.03±0.39 °C compared to 3.72±0.22 °C in females, $p<0.01$). The thermoprotective effect of glucosamine hydrochloride depends on gender, being more pronounced in males. Among the 6 tested COX inhibitors, the most significant thermoprotective effect was observed in the highly selective COX-2 inhibitor celecoxib and the weakly selective central inhibitor paracetamol, warranting in-depth research into their impact on organ and system states following heat trauma, as well as the mechanisms of their thermoprotective action. The thermoprotective effect is not associated with selectivity towards COX (cyclooxygenase): it is not observed in the highly selective COX-2 inhibitor etoricoxib and moderately selective COX-2 inhibitor nimesulide, as well as in non-selective COX inhibitors such as diclofenac sodium and aspirin, which also slows down the recovery of body temperature after heat exposure.*

Conclusions: *A convenient and simple model of acute HT in rats is proposed, demonstrating higher thermosensitivity and a more pronounced thermoprotective effect of glucosamine hydrochloride in males. A significant thermoprotective effect was identified in celecoxib and paracetamol, surpassing other investigated NSAIDs. The mechanism and specific features of this effect require further clarification*

Keywords: *acute heat trauma, hyperthermia, thermoresistance, gender, glucosamine hydrochloride, non-steroidal anti-inflammatory drugs, experiment*

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1. Introduction

Recently, the number of weather-related catastrophes has been increasing. This is primarily associated with anthropogenic influence on the climate, leading to the emergence of extreme situations. Among the most common natural disasters is the impact of extreme environmental temperatures.

Heatwaves, which typically last for several days and sometimes weeks or months, have a significant impact on society, including an increase in the number of diseases and deaths associated with the effect of excessive heat on the body. In addition to direct manifestations in the form of thermal injuries (TI), the course of chronic diseases can worsen, especially in the elderly and children. In Europe, over the last 50 years, the highest number of weather and climate-related deaths (93 % of cases, specifically 148,109 registered fatalities) have been attributed to the influence of extreme temperatures [1]. In 2022, an abnormal increase in temperature covered a large part of the Eurasian continent and North America,

and these manifestations were especially strong in Europe and China. [2]. The study [3] found 62,862 heat-related deaths in Europe in 2022; 61,672 of these deaths occurred between May 30 and September 4. Italy, Spain, Germany and France had the highest female and male heat-related mortality numbers.

High temperatures can lead to heat-related conditions such as hyperthermia, heat exhaustion, syncope, heat oedema, seizures, and heat stroke. Physical activity, especially in hot and humid weather, as well as insufficient fluid intake, can contribute to these conditions. Individuals at high risk include healthy workers in hot environments, firefighters, farmers, and athletes exposed to challenging working conditions and environments. Those with cardiovascular diseases, diabetes, and kidney failure are also at increased risk [4]. Heat-related conditions become especially critical in the context of military actions, creating conditions for acute overheating due to explosions, fires, etc., which is relevant to the current situation in Ukraine.

The pathogenesis of heat-related injuries is based on increased heat production and decreased heat output. Initially, adaptive mechanisms dominate to compensate for overheating. The body attempts to increase heat output, leading to tachycardia, centralization of blood circulation, vasodilation, and increased sweating. Oxidative processes in the body decrease and oxygen consumption is reduced. As body temperature rises, a stress reaction develops, activating the sympathetic-adrenal system, releasing catecholamines and corticosteroids into the blood, disrupting blood circulation, and leading to dehydration, hypoxia, and increased ion and water-soluble vitamin excretion. At the decompensation stage, a breakdown of central and local thermoregulatory mechanisms occurs, resulting in a change in temperature homeostasis. Cardiovascular syndrome is accompanied by increased blood viscosity, activation of lipid peroxidation, membrane destruction, and cell necrosis. The consequence is the massive release of pro-inflammatory cytokines and a systemic inflammatory response, leading to multiple organ failure; without adequate therapy, death occurs [5, 6].

It is important to note that the effectiveness of medications for treating heat-related injuries has not been proven, except for drugs for symptomatic treatment of seizures, oedema, or correction of fluid-electrolyte balance [7]. Considering the mechanism of heat-related injuries and the pathogenic role of inflammation, anti-inflammatory agents, especially non-steroidal anti-inflammatory drugs (NSAIDs), are considered promising thermoprotectors. However, the comparative thermoprotective effectiveness of selective and non-selective cyclooxygenase (COX) inhibitors remains unknown. It is worthwhile to compare them with inhibitors of the arachidonic acid cascade, which lack pronounced anti-inflammatory properties (such as paracetamol). Considering the thermoprotective effect of glucosamine hydrochloride [8, 9], it is important to compare this agent of the polytropic mechanism of anti-inflammatory action with inhibitors of the arachidonic acid cascade.

For these studies, it is important to have an adequate animal model of acute HT. Existing screening models are mostly based on recording the survival time of animals in a high-temperature chamber [10], but they do not meet modern bioethical requirements. It is reasonable to use models of heat-related injuries that do not cause death but induce significant hyperthermia [11]. For this, models with different temperature and time parameters are offered [12]. Therefore, it is important to standardize a convenient and reproducible experimental acute overheating model that causes significant hyperthermia but does not lead to animal death. Such a model should be suitable for studying the mechanisms of adaptation to high environmental temperatures and searching for thermoprotective agents. Additionally, it is relevant to determine the possible dependence of animal sensitivity to acute heat-related injuries and the effectiveness of thermoprotectors on gender. In the case of another extreme temperature environmental impact – cold – male mice were found to be more sensitive than females, and G h/ch induced a more powerful cryoprotective effect in males [13, 14]. It is unknown whether there is sexual dimorphism in thermosensitivity and the effectiveness of thermoprotectors against heat trauma in rats.

The aim of the study is to propose a model of acute HT in rats that does not cause the death of animals, to determine the dependence of thermoresistance on gender, and to compare the effectiveness of the thermoprotective effects of a range of non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and glucosamine hydrochloride in this model.

2. Research design (methodology)

The first stage was aimed to determine the parameters of rats' exposure to high environmental temperatures, causing significant disruption to the animals' temperature homeostasis while adhering to ethical requirements to avoid animal fatalities. Based on the research results discussed in the introduction, a temperature of +55 °C was chosen for this purpose. It was prudent to dynamically monitor the body temperature of the rats to prevent fatal overheating. Post-thermal exposure, the dynamics of temperature recovery were assessed. This allowed for the selection of optimal parameters for a well-reproducible HT.

The second stage involved investigating the thermosensitivity of animals of different genders and assessing the effectiveness of a substance with proven thermoprotective properties G h/ch, depending on gender, using the proposed HT model.

In the third stage, a series of drugs for thermoprotective activity were screened on the proposed HT model. This included inhibitors of the arachidonic acid cascade, which exhibit antipyretic effects in their pharmacological spectrum. Non-selective COX inhibitors such as acetylsalicylic acid (ASA), diclofenac sodium, and paracetamol were chosen, along with moderately selective COX-2 inhibitor nimesulide, and highly selective COX-2 inhibitors celecoxib and etoricoxib. This allowed understanding of the role of selective COX inhibition in the effectiveness of thermoprotection during HT and identifying leaders for further in-depth research.

Stages of the study:

1. Analysis of publications on animal models of acute thermal injury.

2. Selection of optimal parameters for HT modelling and monitoring body temperature dynamics.

3. Determination of possible gender dimorphism in the thermosensitivity of rats and the thermoprotective effect of G h/ch.

4. Comparative study of possible thermoprotective properties of COX inhibitors of different selectivity.

5. Processing and analysis of obtained results.

6. Identification of promising directions for further research.

3. Materials and methods

The study was conducted on 90 white outbred rats of both genders with a weight of 300±50 g. The animals were kept on a standard diet in vivarium conditions with free access to water, constant humidity, and a temperature regime of +22–23 °C. The study complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986, with amendments in 1998) [15], Ukrainian Law No. 3446-IV dated 21.02.2006 "On the Protection of animals from cruel treatment" [16] and the Directive of the European Union 2010/63 EU "On the Protection of Animals Used for Scientific Purposes" [17]. The experiment protocol was reviewed and approved by the bioethics commission of the National University of Pharmacy (NUPh), Kharkiv, Ukraine (Protocol No. 12 dated 10.01.2024).

In a previous experiment, a model of hyperthermia was developed on 8 male rats, which should be described in this part of the article. The animals, housed in individual plastic cages with a grid lid allowing free movement, were placed in a thermostat at +55 °C. Rectal temperature was monitored before the experiment and every 15 minutes during exposure to establish an acceptable degree of hyperthermia without animal fatalities. A medical digital thermometer (Gamma Thermo Base) was used. In this study, described in the following section of the article, the optimal duration of thermal exposure was determined to be 30 minutes based on the criteria for developing significant hyperthermia without animal fatalities.

After choosing an acceptable model of acute HT, the main experiment was performed on 82 rats. The animals were placed in a thermostat at +55 °C for 30 minutes with intermediate control of rectal temperature every 15 minutes. To understand the rate of body temperature recovery, it was monitored every 15 minutes for 1 hour after thermal exposure. The number of rats whose body temperature returned to the initial level and became lower than the initial level was determined. Three series of experiments were conducted on different days, and animals were randomly divided into groups. Each experiment formed its own control group, taking into account the chronopharmacological factor. This approach also made it possible to find out the variability of hyperthermia in the proposed model of acute HT. Control group rats were administered water intragastrically (i/g) through a probe, 10 ml/kg. A number of anti-inflammatory drugs were used for screening thermoprotective efficiency: non-selective COX inhibitors, such as ASA (Aspirin, tablets, "Darnytsia", Ukraine), diclofenac sodium (Diclofenac sodium-KV, capsules, "Kyiv Vitamin Plant", Ukraine); a weak inhibitor of COX-1 and, perhaps, to a greater extent, of COX-2, mainly of central action, which is believed to inhibit COX-3 – a splice variant of COX-1 [18–20] – paracetamol (Paracetamol, capsules, "Zdorovya", Ukraine); moderately selective COX-2 inhibitor nimesulide (Nimesil, granules for preparation of suspension for oral use, "Laboratorios Menarini", Italy); highly selective COX-2 inhibitors celecoxib (Celebrex, tablets, Pfizer, USA) and etoricoxib (Arcoxia, tablets, Merck Sharp & Dohme Idea Inc, USA); anti-inflammatory and metabolitotropic agent – G h/ch (substance, Sigma-Aldrich, Germany). Tablets and powders were crushed in a mortar and suspended in water with the addition of Tween-80. Suspensions were administered i/g in a volume of 2 ml/kg of body weight (0.20 ml per 100 g of animal weight) in a prophylactic mode 60 minutes before HT.

In the first series of experiments, the dependence of the degree of hyperthermia and thermoprotective efficiency of G h/ch on gender was evaluated: group 1 – control pathology (HT), males, n=6; group 2 – control pathology (HT), females, n=6; group 3– G h/ch, 50 mg/kg, males+HT (n=8); group 4 – G h/ch, 50 mg/kg, females+HT (n=8). The dose of G h/ch corresponds to the data [8]. The second and third series were devoted to the screening of potential thermoprotectors among COX inhibitors, and in these experiments, more thermosensitive males were used.

The second series of experiments: 1 group – control pathology (HT), n=6; group 2 – etoricoxib, 7.9 mg/kg+HT (n=8); group 3 – celecoxib, 8.4 mg/kg+HT (n=8); group 4 – diclofenac sodium, 7 mg/kg+HT (n=8).

The third series of experiments: 1 group – control pathology (HT), n=6; group 2 – nimesulide, 6.8 mg/kg+HT (n=6); group 3 – paracetamol, 125 mg/kg+HT (n=6); group 4 – ASA, 25 mg/kg+HT (n=6).

When choosing the doses, we focused on the data on the effective doses of the specified drugs in acute cold injury [21, 22], while equimolar doses were used for nimesulide and coxibs.

The STATISTICA 12.0 program was used to process the results statistically. The reliability of temperature differences within each group in dynamics was determined by the paired Wilcoxon test and between-group differences – by the Mann-Whitney test. The signs taken into account in the alternative form were compared by Fisher's angular transformation (φ). Changes were considered reliable at a significance level of p<0.05. Correlation analysis was used to clarify the relationship between individual indicators.

4. Results

Reasoning of the modelling regime for acute thermal injury in rats.

The average initial body temperature of rats in different groups ranged from 36.1 to 38.1 °C, corresponding to the physiological norm for this species [23].

During a 15-minute exposure at +55 °C, the body temperature of male rats increased on average from 37.15±0.15 °C to 38.07±0.16 °C (by 2.5 %). In the next 15 minutes, it further rose to 40.07±0.43 °C (by 2.92 °C or 7.9 % from the baseline), reaching 41.7 °C in some individuals. The rapid temperature increase in most rats, nearly 3 °C (in some cases, 5 °C), corresponds to the second period of hyperthermia when thermoregulatory capabilities are insufficient to counteract overheating. At the same time, the general condition of the animals was heterogeneous: some rats showed motor restlessness, and others, on the contrary, lethargy. Further thermal exposure of most rats was impractical, as it threatened fatal overheating. 1 rat was found to be thermoresistant, whose body temperature increased by only 1.7 °C or 4 % (from 37.2 °C to 38.7 °C) in 30 minutes.

The results of this experiment support the recommendation of a 30-minute exposure of rats at +55 °C as a hyperthermia model that does not lead to mortality and is ethically acceptable.

Dependence of rats' thermoresistance and the thermoprotective action of glucosamine hydrochloride on gender.

As can be seen from Table 1, in male rats, the body temperature increased more during thermal exposure than in females. The maximum increase in temperature at the 30th minute was 5.03±0.39 °C in males compared to 3.72±0.22 °C in females ($p<0.01$); in females, it was on average 26 % less (Table 2).

The thermoprotective effect of G h/ch was observed in animals of both genders. However, in males, the degree of temperature increase in the presence of G h/ch was 32.8 % less than in the control ($p<0.01$). In females, this difference was 17.7 %, showing only a tendency (Table 2). At the same time, in the male group, the temperature practically reached the initial level after a 30-minute recovery period (Table 1), and in 25 % of the animals, it even decreased compared to the initial level (Table 2). In females, the temperature recovery under the influence of G h/ch occurred somewhat slower.

At the same time, in the group of males on the background of G h/ch after 30 min. during the recovery period, the temperature almost reached the initial level (Table 1), and in 25 % of the animals it even decreased compared to the initial level (Table 2). In females, the recovery of temperature under the influence of G h/ch was somewhat slower.

Screening of arachidonic acid cascade inhibitors for thermoprotective action.

In the series of experiments dedicated to the screening of COX inhibitors, the degree of hyperthermia in control groups (control-1, control-2) is compared with the previous series, indicating the reproducibility of the HT model (Table 3).

Non-selective COX inhibitors diclofenac and ASA practically did not cause a thermoprotective effect: the maximum increase in temperature under the influence of diclofenac was 10.4 % against 11 % in control-1, under the influence of ASA –10.1 % against 9.6 % in control-2, which did not reach a significant level (Table 3). The course of the recovery period (especially in the ASA group) was characterized by a slower decrease in hyperthermia in the recovery period than in the respective control groups. In control, the average body temperature did not have statically significant differences with the initial one after 30 minutes of recovery, and against the background of diclofenac and ASA, it remained significantly higher for the entire 60 minutes observation in the ASA group for 60 minutes of even increased compared to the indicator of 45 min. (Table 3). Also, on the background of ASA, the body temperature during the recovery period did not return to the initial level in any animal, against 33.3 % in the control, $p<0.05$ (Table 4).

Paracetamol, a non-selective COX inhibitor with mainly central action, proved to be an effective thermoprotector: it reduced the increase in temperature by 1.6 times after 30 minutes of heat exposure, after which the recovery of temperature was significantly accelerated – after 30 min it tended to be lower than the initial, and after 60 min was significantly lower by 1.5 % (Table 3), and this effect was observed in 100 % of rats (Table 4).

Table 1

Dynamics of rectal temperature of untreated rats and under the influence of glucosamine hydrochloride during exposure at +55°C and in the recovery period depending on gender ($M\pm m$, Me[Q_{25}; Q_{75}])

Group, number of animals	Initial temperature (°C)	Temperature dynamics during thermal exposure		Temperature dynamics in the recovery period			
		15 min	30 min	15 min	30 min	45 min	60 min
Untreated control, n=6, males	36.65±0.15 36.6 [36.5; 36.9]	39.27±0.14** 39.2 [39.0; 39.5] (+7.2 %)	41.38±0.27**# 41.8 [41.1; 42.2] (+13 %)	39.60±0.22** 39.6 [39.3; 39.8] (+8 %)	38.13±0.14** 38.1 [38.0; 38.3] (+4 %)	37.73±0.19** 37.8 [37.4; 38.0] (+3 %)	37.70±0.31** 37.9 [37.4; 38.2] (+2.9 %)
Glucosamine h/ch, n=8, males	38.10±0.28 38.1 [37.8; 38.6]	40.06±0.32** 39.9 [39.5; 40.6] (+5.1 %)	41.48±0.34**# 41.3 [40.7; 42.2] (+9 %)	39.33±0.29** 39.3 [38.7; 39.6] (+3.2 %)	38.29±0.21 38.2 [37.9; 38.5] (+0.5 %)	–	–
Untreated control, n=6, females	37.25±0.37 37.2 [37.1; 37.6]	39.38±0.14** 39.4 [39.2; 39.6] (+5.7 %)	40.97±0.18**# 41.0 [40.8; 41.2] (+10 %)	39.70±0.22** 39.8 [39.6; 40.0] (+6.5 %)	38.53±0.10** 38.6 [38.5; 38.6] (+3.4 %)	38.35±0.13* 38.4 [38.2; 38.5] (+3 %)	38.27±0.11*38.3 [38.2; 38.4] (+2.7 %)
Glucosamine h/ch, n=8, females	37.69±0.32 37.7 [37.1; 38.2]	39.39±0.21** 39.5 [39.4; 39.7] (+4.5 %)	40.75±0.20*# 40.7 [40.5; 41.1] (+8.1 %)	39.15±0.17** 38.9 [38.9; 39.5] (+3.9 %)	38.39±0.13 38.5 [38.1; 38.7] (+1.9 %)	38.17±0.24 38.3 [38.1; 38.5] (+1.3 %)	38.29±0.21 38.2 [38.0; 38.4] (+1.6 %)

*Note: statistically significant differences compared to the initial state: * – $p<0.05$, ** – $p<0.0$; regarding the indicator for 15 minutes of heat exposure: # – $p<0.01$*

Table 2

Characteristic of the hyperthermic reaction and thermoprotective effect of glucosamine hydrochloride in rats of different genders under conditions of acute heat injury (*M*±*m*)

Group, number of animals	Maximum temperature increase	The number of animals that returned to the initial temperature		The number of animals in which the temperature decreased compared to the initial one	
		abs.	%	abs.	%
Untreated control, *n*=6, males	5.03±0.39	1	16.7	0	0
Glucosamine h/ch, *n*=8, males	3.38±0.27^^	6	75^	2	25^
Untreated control, *n*=6, females	3.72±0.22&	0	0	0	0
Glucosamine h/ch, *n*=8, females	3.06±0.41	5	62.5^	0	0

Note: statistically significant differences compared to the control group of the corresponding gender: ^ – p<0.05, ^^ – p<0.01; & – relative to the group of males (p<0.01).

Table 3

Dynamics of the rectal temperature of male rats under the influence of COX inhibitors during exposure at +55°C and in the recovery period (*M*±*m*, Me[Q_{25}; Q_{75}])

Group, number of animals	Initial temperature (°C)	Temperature dynamics during thermal exposure		Temperature dynamics in the recovery period			
		15 min	30 min	15 min	30 min	45 min	60 min
Untreated control-1, *n*=6	36.95±0.37 36.9 [36.3; 37.3]	38.87±0.40** 38.8 [38.1; 39.4] (+5.2 %)	40.97±0.44**# 40.9 [40.6; 41.7] (+11 %)	39.32±0.27** 39.5 [38.9; 39.7] (+6.4 %)	37.97±0.16* 38.1 [37.7; 38.2] (+2.8 %)	37.47±0.21 37.6 [37.2; 37.7] (+1.4 %)	37.32±0.17 37.3 [37.1; 37.5] (+1 %)
Diclofenac sodium, *n*=8	37.06±0.20 37.1 [36.7; 37.5]	38.89±0.10** 38.8 [38.7; 39.0] (+5 %)	40.91±0.13**# 40.9 [40.7; 41.1] (+10.4 %)	38.58±0.17** 38.4 [38.3; 38.9] (+4.1 %)	37.71±0.16* 37.7 [37.4; 37.8] (+1.8 %)	37.61±0.14* 37.5 [37.4; 38.0] (+1.5 %)	37.66±0.10* 37.7 [37.5; 37.9] (+1.6 %)
Etoricoxib, *n*=8	37.13±0.10 37.2 [37.0; 37.3]	38.90±0.44** 38.9 [38.6; 39.2] (+4.8 %)	40.62±0.36**# 40.6 [39.7; 41.3] (+9.4 %)	38.85±0.39** 38.7 [38.1; 39.5] (+4.6 %)	37.79±0.14** 37.8 [37.6; 38.0] (+1.8 %)	37.54±0.16* 37.6 [37.4; 37.9] (+1.1 %)	37.40±0.15 37.5 [37.3; 37.6] (+0.7 %)
Celecoxib, *n*=8	38.22±0.14 38.2 [38.1; 38.6]	38.97±0.12** 39.1 [38.9; 39.1] (+2 %)	40.10±0.10**# 40.2 [40.1; 40.2] (+5 %)	38.66±0.08* 38.7 [38.7; 38.8] (+1.2 %)	37.72±0.15* 37.8 [37.5; 37.9] (–1.3 %)	37.54±0.19* 37.6 [37.3; 37.8] (–1.8 %)	37.51±0.20* 37.6 [37.3; 37.8] (–2 %)
Untreated control-1, *n*=6	37.33±0.17 37.3 [37.2; 37.4]	39.10±0.21** 39.4 [38.8; 39.4] (+4.7 %)	40.90±0.19**# 40.9 [40.7; 41.1] (+9.6 %)	39.70±0.12** 39.8 [39.6; 39.8] (+6.4 %)	38.42±0.29** 38.2 [38.0; 38.3] (+3 %)	37.90±0.20 37.7 [37.6; 38.2] (+1.5 %)	37.80±0.20 37.7 [37.5; 38.2] (+1.3 %)
Paracetamol, *n*=6	38.28±0.14 38.2 [38.0; 38.5]	38.82±0.29 38.7 [38.4; 38.9] (+1.4 %)	40.62±0.15**# 40.7 [40.6; 40.9] (+6.1 %)	39.18±0.16** 39.2 [38.9; 39.5] (+2.4 %)	38.02±0.09 38.1 [37.9; 38.1] (–0.7 %)	37.92±0.13 37.9 [37.7; 38.1] (–0.9 %)	37.72±0.11* 37.8 [37.5; 37.9] (–1.5 %)
ASA, *n*=6	37.25±0.09 37.4 [37.2; 37.4]	39.25±0.16** 39.1 [39.1; 39.3] (+5.4 %)	41.03±0.20**# 41.2 [41.0; 41.3] (+10.1 %)	39.58±0.10** 39.6 [39.5; 39.6] (+6.3 %)	38.02±0.17** 38.0 [37.8; 38.3] (+2 %)	37.78±0.18* 37.8 [37.5; 38.1] (+1.4 %)	38.20±0.18** 38.2 [37.9; 38.5] (+2.6 %)
Nimesulide, *n*=6	37.85±0.32 38.1 [37.6;38.1]	39.62±0.17** 39.7 [39.5; 39.8] (+4.7 %)	41.23±0.18**# 41.2 [41.1; 41.2] (+9 %)	39.22±0.20** 39.1 [39.0; 39.6] (+3.6 %)	38.18±0.14 38.2 [37.9; 38.3] (+0.9 %)	38.25±0.18 38.3 [38.1; 38.6] (+1 %)	38.32±0.16 38.4 [38.1; 38.5] (+1 %)

Note: statistically significant differences compared to the initial state: – p<0.05, ** – p<0.01; regarding the indicator for 15 minutes of heat exposure: # – p<0.01*

Table 4

Characteristic of the hyperthermic reaction and thermoprotective effect of COX inhibitors in male rats under conditions of acute heat injury (*M*±*m*)

Group, number of animals	Maximum temperature increase	The number of animals that returned to the initial temperature		The number of animals in which the temperature decreased compared to the initial one	
		abs.	%	abs.	%
Untreated control-1, *n*=6	4.02±0.51	2	33.3	1	16.7
Diclofenac sodium, *n*=8	3.85±0.24	4	50	1	12.5
Etoricoxib, *n*=8	3.50±0.40	5	62.5	1	12.5
Celecoxib, *n*=8	1.88±0.12^	8	100^de	8	100^de
Untreated control-2, *n*=6	3.57±0.61	2	33.3	1	16.7
Paracetamol, *n*=6	2.33 ±0.35	6	100^an	6	100^an
ASA, *n*=6	3.78±0.23	0	0^	0	0
Nimesulide, *n*=6	3.38±0.40	1	16.7	0	0

Note: statistically significant differences (p<0.05): ^ – relative to the control group, d – relative to the diclofenac group, e – relative to the etoricoxib group, a – relative to the ASA group, n – relative to the nimesulide group

The moderately selective COX-2 inhibitor nimesulide practically did not reduce the degree of hyperthermia in rats with HT, but it slightly improved the course of recovery, accelerating the decrease in temperature – as early as 30 min, it had no significant differences from the initial (Table 3).

Among the two highly selective COX-2 inhibitors, celecoxib showed powerful thermoprotective properties. It reduced the increase in body temperature during heat exposure by 2.2 times (maximum among all NSAIDs) (5 % vs 11 % in control, $p<0.01$) and provided the most effective reduction in the recovery period: from 30 min it was significantly lower than the initial in 100 % of animals (Tables 3, 4). Etoricoxib had almost no effect on hyperthermia (9.4 % vs. 11 % in controls), and the reduction of temperature in the recovery period only tended to accelerate.

5. Discussion

As a result of the study, a regime of heat exposure of adult rats was chosen, which allows simulation of a non-lethal heat trauma and meets the requirements of bioethics – 30 min at +55 °C. At the same time, the body temperature in different series of studies increases by an average of 8–13 %; in some animals, it reaches 42 °C and higher. The level of hyperthermia achieved corresponds to heat stroke in rats [12]. The correlation analysis shows that the degree of its increase in this model does not depend on the initial body temperature (the correlation coefficient between the initial indicator and after 15 minutes is 0.25, and after 30 minutes is 0.04). But in the dynamics of the development of hyperthermia, a certain regularity was revealed – a direct positive correlation between the increase in temperature for 15 and 30 minutes (r=0.59), which allows predicting the thermosensitivity of rats based on the results of non-traumatic 15-minute heat exposure, selecting animals with the maximum increase (at least by 1 °C). Therefore, already in the course of the experiment, it is possible to exclude a few rats that give an increase in temperature of less than 1 °C during 15 min of thermal exposure, eliminating the need for preliminary thermosensitivity testing.

Male rats were more thermosensitive than females, and the thermoprotective action of G h/ch was more pronounced in them. Similar gender differences have been found in the sensitivity of mice to acute cold exposure and in the frigoprotective effect of G h/ch [13, 14]. Recently, this has also been confirmed for rats [24].

The revealed dimorphism of the thermoprotective effect of G h/ch complements the known gender differences in the effectiveness of many drugs. In particular, in opioid analgesics, NSAIDs, the analgesic effect, as well as the antiplatelet effect of ASA, is more pronounced in females [25].

The results of screening six COX inhibitors for thermoprotective activity revealed two leaders. It is a highly selective COX-2 inhibitor, celecoxib, and a weak non-selective central COX inhibitor, paracetamol. These drugs also significantly accelerated the decrease in temperature during the recovery period: in all animals, it turned out to be even lower than the initial temperature. Unexpectedly, the highly selective COX-2 inhibitor etoricoxib and moderately selective COX-2 inhibitor nimesulide, non-selective COX inhibitors diclofenac sodium and ASA have virtually no thermoprotective effect. It is noteworthy that ASA reliably and nimesulide tend to slow down the decrease in body temperature after HT, worsening the course of the recovery period.

The thermoprotective effect of inhibitors of the arachidonic acid cascade during acute HT obviously does not completely coincide with the well-known antipyretic effect and does not reveal a clear association with a certain selectivity of the effect on COX isozymes. The mechanism of action of coxibs – highly selective inhibition of COX-2 – has been studied in sufficient depth. The presence of a pronounced thermoprotective effect in celecoxib, but not in etoricoxib, may indicate that the mechanisms of the protective effect during overheating of the body and the anti-inflammatory effect dissociate to a certain extent. In a series of studies of the frigoprotective effect of these drugs, both (especially etoricoxib) reduced the severity of hypothermia in acute general cooling, as did diclofenac and, to a lesser extent, ASA, paracetamol [21, 22, 26–29]. Therefore, the thermo- and cold-protective effects of COX inhibitors also dissociate to some extent.

Paracetamol, as mentioned above, has significant features of the mechanism of action. At a low level of arachidonic acid and peroxides, this mainly central analgesic-antipyretic can exhibit the properties of a moderately selective COX-2 inhibitor, which is evidenced by weak antiplatelet activity and the absence of pronounced gastrotoxicity. But with a significant level of arachidonic acid and peroxides, paracetamol loses these properties and, as a result, does not suppress severe inflammation. In addition, unlike both non-selective NSAIDs and selective COX-2 inhibitors, this drug inhibits other peroxidase enzymes, including myeloperoxidase, which promotes the oxidation of paracetamol and reduces the formation of halogenating oxidizers, such as chloroacetic acid, and their role in the pathogenesis of inflammatory processes. The multiplicity of the mechanisms of action of paracetamol also consists in the activation of serotonergic, opioid and cannabinoid systems of the brain [20, 30]. Analysis of these mechanisms suggests that the effect of paracetamol on thermoregulation during acute heat exposure may occur through different pathways.

The results of the study of the thermoprotective effect of HT encourage us to investigate in detail its mechanisms in the leaders of screening – celecoxib and paracetamol, the reasons for its absence in drugs whose analogues have proven to be effective thermoprotectors by the known mechanism of anti-inflammatory and antipyretic action.

Practical Relevance. The results experimentally justify the feasibility of using celecoxib and paracetamol in acute HT.

Study limitation. Only six COX inhibitors were included in the screening.

Further research prospects. Expanding the range of drugs for studying the thermoprotective effect. In-depth investigation of possible gender dimorphism, dose dependence, and mechanisms in the leaders of the screening. Clarification of the influence of the most ef-

fective thermoprotectors on the central nervous system, visceral systems, hemostasis, metabolism (especially energy and water-electrolyte) under the influence of high environmental temperature.

6. Conclusions

1. A convenient and well-reproducible model of acute heat injury in adult rats by exposure at +55 °C for 30 min is proposed. In isolated cases, there are heat-resistant animals, in which during the first 15 min. body temperature rises by less than 1 °C. They are not used for further modelling of thermal injury.

2. Male rats are more sensitive than females to the effect of high environmental temperature, acute heat injury in them is characterized by greater hyperthermia.

3. The thermoprotective effect of glucosamine hydrochloride depends on gender: it is more pronounced in males than in females.

4. Among the 6 tested COX inhibitors, the highest thermoprotective effect was found in the highly selective COX-2 inhibitor celecoxib and the weak non-selective central inhibitor paracetamol, which deserve an in-depth study of the effect on the state of organs and systems during thermal injury, mechanisms of thermoprotective action. The thermoprotective effect is not related to COX selectivity: it is not detected in highly selective COX-2 inhibitor etoricoxib and moderately selective COX-2 inhibitor nimesulide, non-selective COX inhibitors diclofenac sodium and acetylsalicylic acid, which also slows down recovery of body temperature after thermal exposure.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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