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VASCULAR MECHANISMS IN THE FORMATION OF GENDER DIFFERENCES IN THE PROTECTIVE EFFECT OF GLUCOSAMINE IN EXPERIMENTAL COLD INJURY

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The purpose of the study was to assess sex-specific changes in the production of vasoactive molecules during experimental acute cold injury and to investigate endotheliotropic mechanisms that ensure the formation of the body's sex-determining response to the frigoprotective effect of glucosamine. Changes in the production of vasoconstrictor molecules (endothelin-1, adhesion molecules of cranial cellin-1 and vasodilating molecules (H_2S, NO) and markers of oxidative stress (nicotinamide adenine dinucleotide phosphate oxidase, superoxide dismutase) in rats of different sexes and the effect on these parameters of prophylactic and therapeutic administration of glucosamine (50 mg/kg intragastrically) were studied on the model of acute cold injury. It was established that acute cold injury causes an increase in the activity of nicotinamide adenine dinucleotide phosphate oxidase, a decrease in the activity of superoxide dismutase in animals of both sexes, an increase in the content of vasoconstrictors and a decrease in the production of vasodilating molecules, to a greater extent in male animals. The endothelial dysfunction degree and oxidative stress's expressiveness in females were statistically lower. Therapeutic and prophylactic administration of glucosamine reduces the severity of pathological changes in the body of animals caused by acute cold injury to a greater extent in male rats. The degree of expressiveness of sex differences in the indicators studied remains. However, the difference in indicators between animals of different sexes decreases.

Key words: cold injury, gender differences, rats, glucosamine, vasoactive molecules, hydrogen sulfide, nitrogen monoxide, oxidative stress

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СУДИННІ МЕХАНІЗМИ ФОРМУВАННЯ СТАТЕВИХ ВІДМІННОСТЕЙ ЗАХИСНОЇ ДІЇ ГЛЮКОЗАМІНУ ЗА ЕКСПЕРИМЕНТАЛЬНОЇ ХОЛОДОВОЇ ТРАВМИ

У дослідженні оцінено стать-специфічні зміни продукції вазоактивних молекул за експериментальної гострої холодової травми та встановлено ендотеліотропні механізми, що забезпечують формування статевої детермінації відповіді організму на фригопротекторну дію глюкозаміну. На моделі гострої холодової травми досліджено зміни продукції вазоконстрикторних (ендотелін-1, адгезивної молекули судинних клітин-1 та вазодилатуючих (H_2S, NO) молекул та маркерів оксидативного стресу (нікотинамідаденіндинуклеотидфосфат-оксидаза, супероксиддисмутаза) у щурів різної статі та вплив на ці показники профілактично-лікувального введення глюкозаміну (50 мг/кг внутрішньошлунково). Встановлено, що гостра холодова травма викликає підвищення активності нікотинамідаденіндинуклеотидфосфат-оксидази, зниженням активності супероксиддисмутази у тварин обох статей, збільшення вмісту вазоконстрикторних та зменшенням продукції вазодилатуючих молекул, в більшій мірі у тварин чоловічої статі. Ступінь ендотеліальної дисфункції та оксидативного стресу у особин жіночої статі були статистично меншими. Лікувально-профілактичне введення глюкозаміну зменшує виразність патологічних змін в організмі тварин, спричинені гострою холодовою травмою, в більшій мірі у самців щурів. Ступінь виразності статевих відмінностей показників, що вивчались, залишається, однак різниця в показниках між тваринами різної статі зменшується.

Ключові слова: холодова травма, статеві відмінності, щури, глюкозамін, вазоактивні молекули, гідроген сульфід, нітроген моноксид, оксидативний стрес

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Cold injury currently remains a vital problem today both in our country and in almost all parts of our planet. According to medical reports, the average hospitalisation rate of cold injury victims is 6.0 per 100,000 population. Of all admissions to thermal trauma units, cold-affected patients account for 3 to 30 % [1]. The average duration of treatment for frostbite is more than twice as long as the duration of therapy for patients with burns. Disability due to frostbite ranges from 20 to 48 %, and for deep injury – 70 to 94 %, professional capacity after treatment is maintained by an average of 59 % of recoveries.

During military operations, frostbite becomes extremely important and constitutes a significant percentage of sanitary losses. Today, there are still many unexplored factors that can affect cold resistance. These include age, gender, and even nationality. According to most of the data in the literature, frostbite is

more common in the male sex. This is confirmed at the population level in [7, 13]. However, in some places in the literature, it is possible to note works in which it is admitted that cases of frostbite were observed more often in women than in men [12]. Such controversial data regarding the gender determination of this pathology justify the expediency of in-depth studies of the specifics of the development of cold injury in males and females, as well as gender differences in the response to drug treatment of this condition.

Pathogenetic processes that occur when the body is cooled develop according to the neurohumoral theory when changes in tissues during thawing are the result of a combination of factors such as pain, toxaemia, prethrombotic state, impaired capillary blood circulation, infection, etc. Disruption of microcirculation plays a special role in pathogenesis. As a result of hypoxia and the direct effect of cold, serotonin, kinins, histamine, and their metabolites accumulate in the tissues, which cause pathological changes in the vascular bed [5, 6]. An important place in the mechanisms of the development of pathology during frostbite is occupied by processes of alteration of the endothelium.

A wide range of medicines are used in the treatment of cold injury. Among them, great attention is paid to analgesic, anti-inflammatory and vasoactive drugs [10]. Experimental and clinical studies show that glucosamine showed an effective frigoprotective effect [1]. Despite the in-depth analysis of the mechanisms and features of its protective effect under conditions of acute cold injury, the sexual dimorphism of the body's response to its frigoprotective effect is poorly studied.

The purpose of the study was to assess sex-specific changes in the production of vasoactive molecules during experimental acute cold injury and to investigate endotheliotropic mechanisms that ensure the formation of the body's sex-determining response to the frigoprotective effect of glucosamine.

Materials and methods. Experiments were performed on 60 sexually mature (3 months) male and female Wistar rats obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. The study was carried out in the autumn, starting early in the morning. In the experiment, female animals were taken in the phase of proestrus, which was determined using vaginal smears. All stages of research were carried out in compliance with International requirements for humane treatment of animals, following the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" and current laws of Ukraine and approved by the bioethics commission of National Pirogov Memorial Medical University (protocol No. 10 dated 02.12.2022). Animals were kept in standard vivarium conditions and had access to food, water *ad libitum*, and a 12-hour day/night regime.

According to the purpose, the experimental animals were divided into the following: Group 1 – male and female rats treated with acute cold injury (ACI); Group 2 – sexually mature animals of both sexes who were intragastrically administered glucosamine hydrochloride (50 mg/kg) 60 minutes before ACI modelling and 3 days after it (Sigma, USA); Group 3 – control group sexually mature animals of both sexes who received equal-volume amounts of solvent instead of the test substance.

ACI modelling was performed by methodical recommendations [3]. Male and female rats were placed in plastic transparent boxes measuring 10x15x20 cm, which do not restrict access to air and the mobility of animals, and kept in a refrigerator at -18ºC for 2 hours. Determination of biochemical parameters in the blood was carried out on the 3rd day after exposure to cold. Animals were euthanised by dislocation of the cervical vertebrae.

The content of endothelin-1 (ET-1) in blood serum was determined by the immunoenzymatic test using the commercial kit “Rat ET-1 (Endothelin 1) ELISA Kit” (Elabscience Biotechnology Inc., USA) by the manufacturer's instructions on the analyser STAT FAX 303/PLUS. The content of NO in blood serum was determined by a colorimetric analysis at a 550 nm wavelength using a "Nitric Oxide (NO) Colorimetric Assay Kit" commercial kit ("Elabscience Biotechnology Inc., USA). The content of H_2S in serum was determined by the reaction of thionine formation using n-phenylenediamine [4]. The content of vascular cell adhesion molecule-1 (sVCAM-1) in blood serum was determined by the immunoenzymatic method using the "sVCAM-1 ELISA KIT" kit (Diaclone, France) according to the manufacturer's instructions. Superoxide dismutase (SOD) activity in blood was assayed using a kit (Cayman Chemical Co., Ann Arbor, MI, USA) following the manufacturer's standard procedures, NADPH oxidase – by the degree of absorption of NADPH at 340 nm [9].

Statistical processing of the obtained results was done using standard methods using the program "Statistica SPSS 10.0 for Windows" (license number 305147890). The obtained results were presented in the form of M±m. The Shapiro-Wilk test was used to check the normality test. The reliability assessment of the difference in mean indicators was done using the Student's t-test (for a normal distribution) and the

Mann-Whitney U-test (if the distribution deviates from the normal). Differences at $p<0.05$ were considered statistically significant.

Results of the study and their discussion. The obtained results demonstrated gender differences in the molecules' content that ensured the endothelium's normal function in the control group of experimental animals. At the same time, the content of endothelin-1 (the most potent vasoconstrictor in the organism) differed slightly between individuals of different sexes ($p>0.05$), and the quantitative index of the endothelial dysfunction marker sVCAM-1 in females was statistically lower (by 13.3 % than in males control animals. On the other hand, the content of low-molecular-weight vasodilating molecules H_2S and NO was statistically significantly higher in females (by 17.4 and 50.5 %, respectively) compared to males (Table 1).

Table 1

The content of vasoactive mediators and soluble vascular cell adhesion molecules in male and female rats under acute general cooling and on the background of glucosamine correction (M±m, n=10)

No.	Parameter	Males	Females
Control group			
1	Endothelin-1, ng/L	62.4±2.15	59.8±2.03
2	H_2S, μmol/L	75.2±2.50	88.3±2.78*
3	NO, μmol/L	30.5±1.54	45.9±1.69*
4	sVCAM-1, ng/mL	520±15.4	451±16.4*
Acute general cooling			
1	Endothelin-1, ng/L	90.5±3.01#	76.5±2.59*#
2	H_2S, μmol/L	52.6±1.95#	70.7±2.15*#
3	NO, μmol/L	20.8±1.44#	34.4±1.55*#
4	sVCAM-1, ng/mL	686±17.8#	563±16.3*#
Acute general cooling + glucosamine			
1	Endothelin-1, ng/L	87.6±2.94#	73.2±2.48*#
2	H_2S, μmol/L	64.6±2.13#$	79.3±2.48*#$
3	NO, μmol/L	25.5±1.56#$	39.5±1.39*#$
4	sVCAM-1, ng/mL	578±16.9#$	501±15.9*#$

Notes: 1. * – $p<0.05$ between males and females within the group; 2. # – $p<0.05$ relative to the control group; 3. $ – $p<0.05$ relative to untreated animals with acute general cooling.

Acute cold injury causes a whole series of molecular disorders, with an increase in the content of vasoconstrictors in the blood and a decrease in the level of vasodilating molecules. These disorders were more pronounced in male animals, as H_2S and NO content on Day 3 after ACI decreased by 30.1 and 31.8 %, respectively ($p<0.05$), compared to 19.9 and 25.1 % in individuals of the opposite sex. The content of endothelin-1 in male animals increased by 45.0 % and in female animals by 27.9 % ($p<0.05$). Activation of endothelin-1 synthesis is probably a response to endotheliocyte damage by toxins during the development of the inflammatory process in the body in response to ACI. According to modern concepts, the endothelium is considered not only as a blood-tissue barrier but also as a neuroendocrine organ that performs several functions: regulation of thrombogenicity and resistance, vascular tone, and leukocyte adhesion. Vascular endothelial cells are susceptible to low temperatures due to their high degree of differentiation. This can result in severe disorders of the blood clotting system, fibrinolysis, and tissue metabolism disorders.

The development of endothelial dysfunction after ACI also confirms the increase in the level of a vital adhesion molecule (sVCAM-1). Its content in the blood of males increased by 31.9 % ($p<0.05$) and in females by 24.8 % ($p<0.05$), relative to the control. Under these conditions, the deepening of gender differences in the functioning of the endothelium is noted: in the ACI group, the level of sVCAM-1 in the blood of females became lower by 17.9 % ($p<0.05$), compared to that in males.

Therapeutic and prophylactic administration of glucosamine caused a significant decrease in the imbalance in the vasoconstrictor-vasodilator system caused by the action of the cold factor in animals of both sexes: an increase in the content of endothelin-1 and sVCAM-1, as well as the decrease in the levels of NO, H_2S, was smaller, compared to animals with ACI without correction. However, a complete return of indicators to the level of control animals was not noted. It turned out to be interesting the fact that the normalising effect of glucosamine on the studied indicators was more clearly manifested in male animals, except sVCAM-1, the content of which decreased in males and females almost equally – by 11.15 and

11.08 %, respectively (p<0.05) relative to rats with ACI. The gender difference in the level of all indicators in the blood of animals was preserved under these conditions of the experiment.

The state of the oxidant-antioxidant system in the bodies of experimental animals was assessed by the activity of NADPH oxidase, which is the main producer of superoxide anion and superoxide dismutase (Fig. 1).

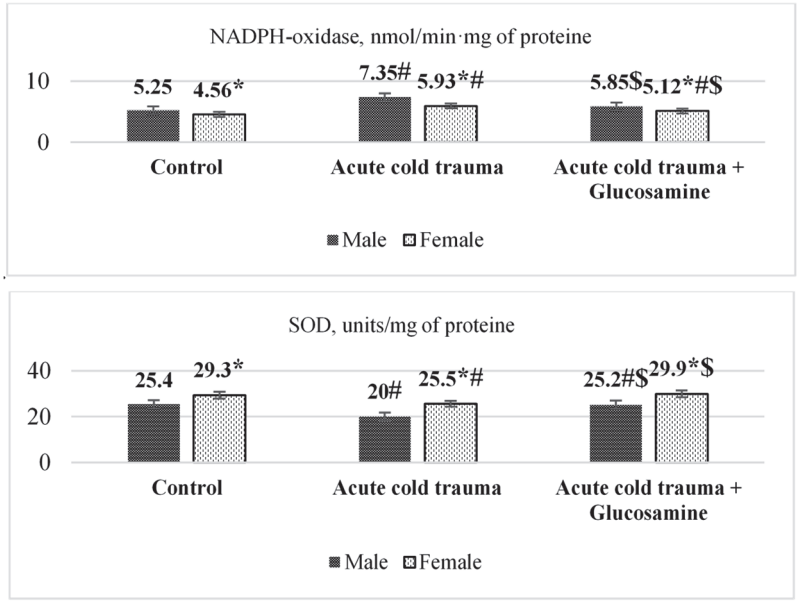

Fig. 1. The state of pro- and antioxidant enzyme systems of blood serum in male and female rats during acute general cooling and against the background of correction with glucosamine (M±m, n=10) Notes: 1. * – p<0.05 between males and females within the group; 2. # – p<0.05 relative to the control group; 3. $ – p<0.05 relative to untreated animals with acute general cooling.

It was established that the activity of NADPH oxidase in the blood serum of males is significantly higher, and the activity of SOD is lower, respectively, by 13.14 and 15.34 % (p<0.05) than in females.

Acute cold injury caused the activation of oxidative stress in animals of both sexes, as evidenced by the elevation of the pro-oxidant enzyme NADPH-oxidase and the depression of the activity of the antioxidant protection enzyme SOD. However, in males, the activity of these parameters changed probably more: for example, the increase of NADPH oxidase in males was by 43.55 %, while in females – by 30.4 %, and the decrease in SOD activity was 21.25 and 12.96 % in males and females, respectively (p<0.05). Gender differences in the predominance of oxidative processes in male animals were preserved.

Prophylactic and therapeutic administration of glucosamine significantly reduced the manifestations of oxidative stress. Thus, compared to untreated animals, NADPH oxidase activity increased by only 11.42 and 12.28 % in males and females, respectively, and this value did not return to baseline. On the other hand, the activity of SOD, on the contrary, probably increased by 26.0 and 17.3 %, respectively, in males and females compared to animals with ACI without correction. The value of this index almost reached the level of control animals. That is, glucosamine showed a powerful antioxidant effect and prevented the development of oxidative stress in animals with ACI, and its impact on this link of the pathogenesis of the pathological condition did not have a gender difference. The antioxidant effect of glucosamine is also confirmed in earlier studies under conditions of general cooling and local cold injury [2].

The conducted studies proved the existence of gender differences in indices of the functional state of the endothelium and oxidant-antioxidant balance in rats. In the blood serum of males, a likely higher activity of NADPH oxidase is noted, accompanied by a more significant activity of lipid and protein peroxidation processes than in females.

Acute general cooling is accompanied by an increase in the activity of NADPH-oxidase, a decrease in the activity of SOD in animals of both sexes, an increase in the content of vasoconstrictors (endothelin-1 and sVCAM-1), and a reduction in the production of vasodilating molecules (NO and H_2S), which occur in a sex-specific manner – to a greater extent in male animals. On the other hand, the degree of endothelial dysfunction and the expressiveness of oxidative stress in females were statistically lower.

Therapeutic and prophylactic administration of glucosamine significantly reduces the severity of pathological changes in the body of animals caused by ACI, and its protective effect is more significantly manifested in male rats. The degree of expressiveness of sex differences in the indices studied remains. However, the difference in indices between animals of different sexes decreases.

In our opinion, the main role in the sexual dimorphism of the vascular response to cold injury and its correction with glucosamine belongs to gonadal hormones, which are directly involved in the regulation of the expression or modulation of the activity of enzymes involved in the formation of vasoactive molecules. In particular, the greater resistance of female individuals to the effects of cold is due to the

positive impact of ovarian estrogens on vascular tone [11], which is realised mainly through non-genomic mechanisms, namely through the implications for exonuclear estrogen receptors and ion channels [8]. In addition, estrogens increase the production of vasodilator molecules such as hydrogen sulfide and reduce the production of vasoconstrictor derivatives of cyclooxygenase and other powerful vasoconstrictors, such as endothelin-1 [14]. Gender differences in the activity of pro-antioxidant enzymes and the activity of free radical oxidation of lipids and proteins are also due to the multidirectional influence of sex hormones on these processes. In particular, according to the literature, estrogens have an antioxidant effect, while the intensity of oxidative stress is more significant in males [15].

Conclusion

Studies have shown that the different content of vasoactive molecules in male and female rats is an essential factor that determines the sex differences of different sensitivities of the animal body to the action of pathogenic factors and the protective effect of glucosamine.

The obtained results prove the practicality of further in-depth research in this direction, which will permit the development of practical approaches to the prevention and treatment of cold injury in persons of different sexes and the carry out of personalised pharmacotherapy of cold injury with glucosamine preparations and other frigoprotectors.

References

1. Bondariev YeV. Eksperymentalne obgruntuvannia optymizatsiyi profilaktyky ta likuvannia kholodovoyi travmy zasobamy metabolitotropnoyi ta protyzapalnoyi diyi [dysertatsiya]. Kharkiv: Nats. Farm. un-t. 2020; 45 s. [in Ukrainian]

2. Bondariev YeV. Fryhoprotektorni ta antyoksydantni vlastyvosti preparativ hliukozaminu ta atsetylsalitsylovoyi kysloty v umovakh hostroyi lokalnoyi kholodovoyi travmy. Ukrainskyi biofarmatsevtychnyi zhurnal. 2018; 1(54):18–24. [in Ukrainian]

3. Bondariev YeV, Shtryhol SIu, Drohovoz SM, Shchokina KH. Kholodova travma: doklinichne vyvchennia likarskykh preparativ z fryhoprotektornymy vlastyvostiamy. Metodychni rekomendatsii MOZ Ukrainy, m. Kharkiv. Natsionalnyi farmatsevychnyi universytet. 2018; 35 s. [in Ukrainian]

4. Melnyk AV. Statevi osoblyvosti rivnia hidrohen sulfidu v syrovattsi krovi shchuriv. Visnyk morfolohiyi. 2017; 1(23):7–9. [in Ukrainian]

5. Pelypenko OV, Shepitko VI, Pelypenko LB. Morfofunktsionalna kharakterystyka mikrotsyrkuliatornoho rusla subkhondralnoyi kistky pry vvedenni kriokonservovanoyi platsenty na tli aseptychnoho zapalennia suhlobu u shchuriv. Svit medytsyny ta biolohiyi. 2019; 2(68):187–191. [in Ukrainian]

6. Anand P, Privitera R, Yiangou Y, Donatien P, Birch R, Misra P. Trench Foot or Non-Freezing Cold Injury As a Painful Vaso-Neuropathy: Clinical and Skin Biopsy Assessments. Front Neurol. 2017; 8:514. https://doi.org/10.3389/fneur.2017.00514.

7. Endorf FW, Nygaard RM. High Cost and Resource Utilization of Frostbite Readmissions in the United States. J Burn Care Res. 2021 Sep 30;42(5):857–864 https://doi.org/10.1093/jbcr/irab076.

8. Hristov KL, Parajuli SP, Provence A, Rovner ES, Petkov GV. Nongenomic modulation of the large conductance voltage- and Ca^{2+}-activated K^+ channels by estrogen: A novel regulatory mechanism in human detrusor smooth muscle. Physiol Rep. 2017 Jul;5(14):e13351. doi: 10.14814/phy2.13351

9. Ma J, Li Y, Yang X, Liu K, Zhang X, Zuo X, Ye R, et al. Signaling pathways in vascular function and hypertension: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 2023 Apr 20;8(1):168. doi: 10.1038/s41392-023-01430-7.

10. McIntosh SE, Freer L, Grissom CK, Auerbach PS, Rodway G W, Cochran A et al. Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Frostbite: 2019 Update. Wilderness & Environmental Medicine. 2019; 30(4S): S19eS32. DOI: https://doi.org/10.1016/j.wem.2019.05.002.

11. Moreau KL. Intersection between gonadal function and vascular aging in women. J Appl Physiol (1985). 2018 Dec 1;125(6):1881–1887. doi: 10.1152/japplphysiol.00117.2018

12. Nagarajan S. Update: Cold weather injuries, active and reserve components, U.S. Armed Forces, July 2010-June 2015. MSMR. 2015 Oct;22(10):7–12.

13. Nygaard R. M., Endorf F. W. Frostbite vs Burns: Increased Cost of Care and Use of Hospital Resources J Burn Care Res. 2018 Aug 17;39(5):676–679.

14. Teoh JP, Li X, Simoncini T, Zhu D, Fu X. Estrogen-Mediated Gaseous Signaling Molecules in Cardiovascular Disease. Trends Endocrinol Metab. 2020 Oct;31(10):773–784. doi: 10.1016/j.tem.2020.06.001

15. Xiang D, Liu Y, Zhou S, Zhou E, Wang Y. Protective Effects of Estrogen on Cardiovascular Disease Mediated by Oxidative Stress. Oxid Med Cell Longev. 2021 Jun 28;2021:5523516. doi: 10.1155/2021/5523516.

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