UDC 620.3:661.693:546.763:547

REACTIONS OF PHYSIOLOGICAL SYSTEMS IN THE ORGANISMS OF RATS TO FEEDING WITH LOW AND HIGH DOSES OF GERMANIUM "NANOAQUACITRATE"

R. S. Fedoruk, O. P. Dolaychuk, I. I. Kovalchuk, M. M. Tsap

Institute of Animal Biology, NAAS; 38, V. Stusa Str., Lviv, Ukraine, 79034

e-mail: ecology@inenbiol.com.ua

Received on September 17, 2015

Aim. To study the changes in the condition of specific functional systems in the organisms of rats in the period of feeding them with different doses of germanium "nanoaquacitrate". **Methods.** The automatic veterinary hematological analyzer Orphee Mythic 18 Vet was used to determine hematological indices. The colorimetric methods were used to study the reactivity of the organism as regards the content of average weight molecules, glycoproteins, monosaccharides of their carbohydrate components in blood serum and as regards its phagocytic activity; hydroperoxides of lipids and TBA-active metabolites were determined. The physiological methods of estimating 10-day dynamics of changes in the body weight of living rats and the weight of the heart, liver, kidneys, pancreas, spleen after slaughter with the determination of their masometric coefficients were used. **Results.** Intergroup differences in hematological indices were revealed on the background of the increase in the number of erythrocytes, hemoglobin, platelets and erythrocyte volume fraction (EVF) along with the decrease in the number of leukocytes and their separate forms in the blood of rats of experimental groups, which was reliably expressed in experimental group III (2.5 mg GeNAC per 1 kg of b.w.). A similar tendency was found for the content of sialic acids and protein-bound hexoses in the blood of animals of group III, TBA-active metabolites – in group II (2.5 μg GeNAC/kg of b.w.), and average weight molecules – groups II and III. The response of the organisms of rats depending on the intensity of their growth confirms more expressed biological impact of Ge in the dose of 2.5 mg/kg b.w. (with the preservation of a higher level of body weight indices) by 4.4–6.9 % and the weight of internal organs – by 5.2–7.5 % during the experiment period. **Conclusions.** The physiological response of the immune, antioxidant and homeostasis functional systems of the organism of rats, their growth and development as regards the body weight and internal organs on feeding with low and high doses of Ge citrate stipulate unlike-directed changes in hematological and physiologically biochemical indices and is characterized by the following: 1) higher ($p \leq 0.05$) level of erythrocytes, EVF, leukocytes in the blood of animals of group III, its phagocytic activity and the content of ceruloplasmin – in group II; 2) lower level of indices of the organism reactivity – the content of average weight molecules in the blood of animals of groups II and III ($p \leq 0.05$), sialic acids, protein-bound hexoses as well as the intensity of peroxide oxidation of lipids by the content of TBA-active metabolites and lipid hydroperoxides in group II; 3) higher intensity of growth and development of the organisms of animals of group II by 3.2–4.0 % during the first 20 days, and group III – by 2.0–6.9 % during 40 days of feeding with Ge citrate as well as the weight of studied internal organs by 1.0–2.5 and 2.3–7.5 % in groups II and III respectively.

Keywords: germanium "nanoaquacitrate", glycoproteins, hematological indices, peroxidation of lipids, rats, growth and development.

DOI: 10.15407/agrisp2.03.015

INTRODUCTION

It is known that microelement Ge stipulates a number of physiological effects, the most studied ones among which are the immunomodulatory effect with the activation of γ-interferon production, transporting and transfer of O_2 with the reduced hypoxia on the tissue level; it decreases the harmful effect of hydrogen ions on cells, activating its interaction with O_2, and inhibits the growth of tumors and metastasis [1−3]. As for the daily requirement of Ge, different amounts are

recommended – from 0.4–1 mg [3] and 8–10 mg [4] to 100–300 mg [5], whereas the daily input with food is 0.4–1.5 mg [3]. It was proven that the most physiologically active substances are Ge compounds, containing sesquioxides. Organic compounds of Ge are remarkable for the most expressed ability of transporting O_2 to the tissues and ensuring its interaction with H^+, which stimulates the elaboration of medical and preventive preparations, based on organic compounds of Ge. The first medical preparation of this kind, Germanium 132, was created in Japan. In 2000 a dietary supplement Germavit was registered; in addition to Ge it contains vitamins, antioxidants and microelements. The preparation Eniogerm was synthesized using the combination with the citric acid. Oral supplementation of organic compounds of Ge determines the increase in its content in the tissues of the stomach, small intestine, bone marrow, spleen and blood. It is proven that a high content of Ge in the stomach and intestines may testify to the prolongation of the process of its absorption in blood [5]. The data of other authors [2, 3] demonstrate a high level of Ge absorption in the organism (about 95 %) and its relatively even distribution in the organs and tissues, in particular, both extracellular and intracellular structures.

As a microelement in the dose of up to 100 mg, Ge is not toxic and 90 % of it are removed from the organism with urine. However, non-organic compounds, in particular Ge oxides, obtained in the process of chemical synthesis, may have toxic impact on the organisms of humans and animals [5, 6]. Therefore, there is a search for new water-soluble organic compounds of Ge using edible acids (oxalic, citric, malic, tartaric acids) to obtain their complex salts–germanates. At present the most promising method of obtaining "organic" Ge is an efficient method, developed by Kosinov and Kaplunenko in Ukraine [7] using nanotechnologies, which ensures obtaining aqueous solutions of Ge carboxylates of ultra-high chemical purity. However, the study of the biological effect of these compounds is just beginning [2, 8].

The aim of the study was to investigate the changes in the condition of specific functional systems in the organisms of rats in the period of feeding them with different doses of germanium "nanoaquacitrate".

MATERIALS AND METHODS

The experiment was conducted in the vivarium of the Institute of Animal Biology NAAS using white laboratory male rats, 180–200 g b.w., at the age of 4 months, divided into three groups of 5 animals each by the principle of analogues. Group I is control, it received balanced standard ration (SR) with granulated mixed feed and unrestricted water supply during the whole period of studies; group II – experimental group, in addition to SR and drinking water it consumed germanium "nanoaquacitrate" (GeNAC), obtained using nanotechnologies [7], in the amount of 2.5 μg GeNAC per 1 kg of b.w. (0.45–0.55 μg Ge per one animal per 1 day); group III – SR + 2.5 mg GeNAC/kg of b.w. (450–550 μg Ge per one animal per 1 day). Feeding male rats of experimental groups II and III with GeNAC solution was carried out for 40 days with daily group control of the amount of consumed water and the calculation of Ge, received with it. After the experiment the animals were decapitated under light chloroform anesthesia for the withdrawal of blood and internal organs and conducting physiological and biochemical studies. The manipulations were performed with no violations of the norms of humane treatment of laboratory animals, in compliance with common bioethical norms and the law "On Protection of Animals from Cruelty", dated February 21, 2006, and in accordance to international provisions on conducting experimental work in compliance to the European Convention for the Protection of Vertebrate Animals [9].

During the preliminary (10 days) and experimental (40 days) periods the indices of body weight, behavior and consumption of feed were controlled. The blood samples were studied as regards the amount of hemoglobin, erythrocytes, leukocytes, lymphocytes, monocytes, granulocytes, platelets, EVF, glycoproteins – ceruloplasmin and their monosaccharides – sialic acids, protein-bound hexoses, average weight molecules (AWM), lipid hydroperoxides (LHP), TBA-active metabolites, phagocytic activity (PA) and phagocytic number (PN) [10]. During the slaughter period the masometric indices of internal organs (heart, kidneys, liver, spleen, pancreas) and the coefficients of their weight against the body weight were determined [11]. The digital material obtained was processed by the method of variation statistics using the Student's criterion. The arithmetic mean values (M) and the deviations of arithmetic mean values ($\pm m$) were calculated. The changes were deemed probable at $P < 0.05$. The estimates were made using MS Excel program.

RESULTS AND DISCUSSION

The analysis of hematological indices expressed reliably the impact of a higher dose of Ge on the number of blood cells and other clinical indices of blood (Ta-

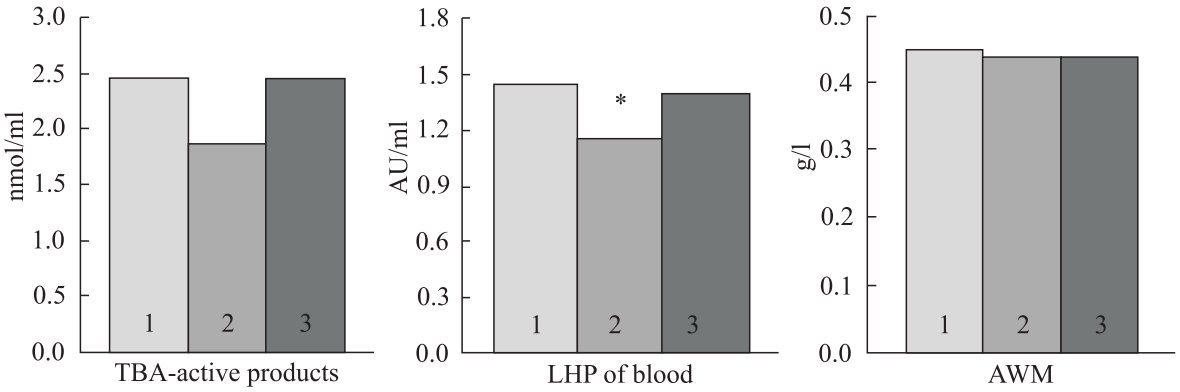

Fig. 1. The indices of the oxidation processes (LHP – lipid hydroperoxides) and the content of average weight molecules (AWM) in the blood of rats: 1 – group I, SR (standard ration); 2 – group II, SR + 2.5 μg Ge; 3 – group III, SR + 2.5 mg Ge. The difference is statistically reliable compared to group I at *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

ble 1). A higher level of erythrocytes, compared to the control group (I), was registered in the blood of rats of group III, which conditioned the increase in EVF ($p \leq \leq 0.05$). Noteworthy is also the increase of the hemoglobin content by 8 % and the number of platelets by 22 % in the blood of animals of this group, but the difference is not reliable. The absence of intergroup differences by hematological indices for animals of group II may indicate weakly expressed physiological effect of GeNAC in the amount of 2.5 μg Ge/kg of b.w. However, the increase in these indices in blood testifies to certain correcting effect of Ge on the supply of O_2 for organs and tissues and the hemostasis of the organism of rats in group II, which is mentioned in other publications [2, 3, 5, 12]. It is confirmed by a smaller amount of leukocytes and their fractions – lymphocytes, monocytes and granulocytes in the blood of rats of both experimental groups, but reliable differences are determined only in group III. A lower level of lymphocytes, monocytes and granulocytes in the blood of animals of experimental groups testifies to the normalizing effect of both applied amounts of germanium on the functional condition of the immune system. However, a more expressed effect on the activity of this system is registered for animals of group III, which is proven by a reliably lower level of monocytes and granulocytes in their blood.

The corrective effect of both applied doses of Ge on the immune system is indicated by higher indices of phagocytic activity of blood and lower indices of AWM content in rats of both group II ($p \leq 0.05$) and group III (but it is not reliable) (Table 2, Fig. 1). At the same time the content of glycoprotein components

Table 1. The hematological indices of the organism of rats fed with the solution of germanium citrate of different concentration ($M \pm m$, $n = 4–5$)

Index	Group		
	I–control	II–2.5 μg Ge	III–2.5 mg Ge
Erythrocytes, 10^{12}/l	7.6 ± 0.18	7.6 ± 0.08	8.5 ± 0.20*
Hemoglobin, g/l	129.4 ± 2.25	127.4 ± 2.36	139.8 ± 4.07
Erythrocyte volume fraction (EVF), l/l	0.39 ± 0.007	0.39 ± 0.004	0.43 ± 0.011*
Leukocytes, 10^9/l	8.0 ± 0.75	6.4 ± 0.36	5.3 ± 0.50*
Lymphocytes, 10^9/l	5.4 ± 0.73	4.5 ± 0.40	3.9 ± 0.39
Monocytes, 10^9/l	0.6 ± 0.09	0.5 ± 0.05	0.4 ± 0.03*
Granulocytes, 10^9/l	1.8 ± 0.30	1.6 ± 0.30	1.0 ± 0.17*
Platelets, 10^9/l	377.4 ± 73.7	404.6 ± 89.4	462.8 ± 52.6

Note. The difference in this and the following Tables is statistically reliable compared to group I at *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

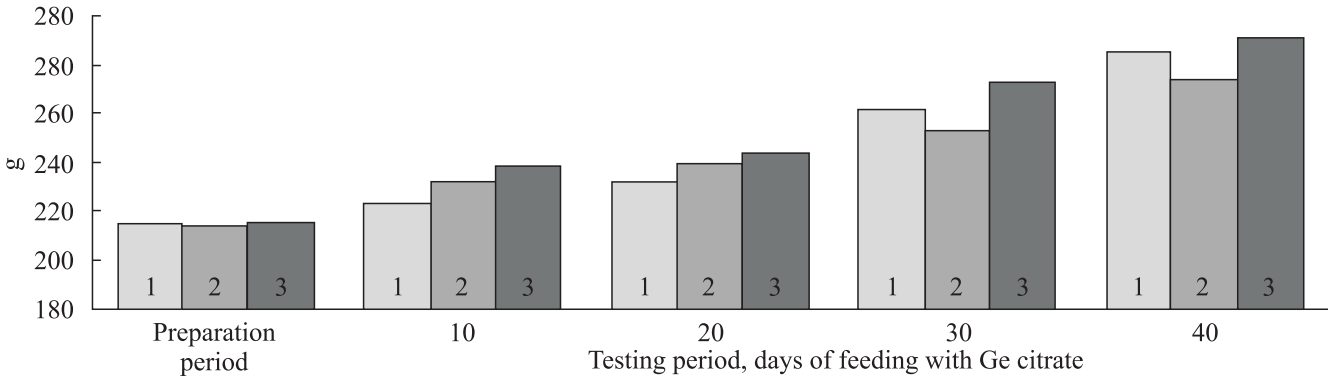

Fig. 2. Age dynamics of the body weight of rats: 1 – group I, SR (standard ration); 2 – group II, SR + 2.5 μg Ge; 3 – group III, SR + 2.5 mg Ge

in the blood of rats of group II increased, and that of group III decreased compared to the control.

It may indicate uneven effect of low and high doses of Ge citrate on the biosynthesis and content of specific immunobiological components in blood, in particular, glycoprotein components, which is reflected by different levels of ceruloplasmin, sialic acids and protein-bound hexoses, for animals of groups II and III. The studies of other authors did not reveal any significant effect of GeNAC on the production of seromucoids in the organism of rats [2]. However, the effect of this compound on the organism of chickens determines the decrease in the synthesis of seromucoids, which is related to the decrease in the immunosuppressive load on the immune system [2]. A similarly directed immunobiological reaction of the organism, aimed at enhancing its resistance with the increase in the level of sialic acids and ceruloplasmin, but with the decrease of protein-bound hexoses in blood, was achieved in the experiment of feeding rats with citrates of Ge, Cr, and Se and citrates of Ge and Cr [12]. Obviously, the physiological effect of Ge citrates in the organism of rats on the concentration of monosaccharides of carbohydrate components of glycoproteins is decisive. It may have significant effect on non-specific resistance of the organism of animals, as it was proven that glycoproteins participate in the functioning of the immune system and the formation of antioxidant protection of the organism [2, 12, 13].

The analysis of the physiological reaction of the oxidant system of the organism of rats as regards the content of TBA-active metabolites and LHP in blood indicates a more expressed impact of the low dose of Ge in group II on the decrease of the intensity of peroxide oxidation of lipids (POL) compared to that of the high dose – in group III (Fig. 1). It is remarkable that the microamount of Ge reliably decreases the content of these metabolites in the blood of rats of group II, whereas a high dose keeps them on the level of the control group.

The studies on chickens determined a higher efficiency of the effect of a low dose (5 μg/chicken) of GeNAC on the decrease in POL intensity in blood, compared to the high dose, – 10 μg/chicken [2]. It is evident that the feeding with 2.5 μg Ge/kg of body weight corrects the reaction of the oxidant/antioxidant system, where-

Table 2. The content of glycoproteins in blood and its phagocytic activity in rats fed with the solution of germanium citrate of different concentration ($M \pm m$, $n = 5$).

Index	Group		
	I–control	II–2.5 μg Ge	III–2.5 mg Ge
Sialic acids, c.u.	267.8 ± 7.91	270.4 ± 6.00	241.6 ± 7.48*
Ceruloplasmin, c.u.	365.2 ± 17.19	439.6 ± 19.14*	341.0 ± 13.10
Protein-bound hexoses, g/l	2.69 ± 0.087	2.74 ± 0.094	2.32 ± 0.091*
Phagocytic activity, %	49.00 ± 1.79	55.20 ± 1.93*	52.60 ± 1.96
Phagocytic number	4.83 ± 0.27	6.20 ± 0.42*	6.03 ± 0.57

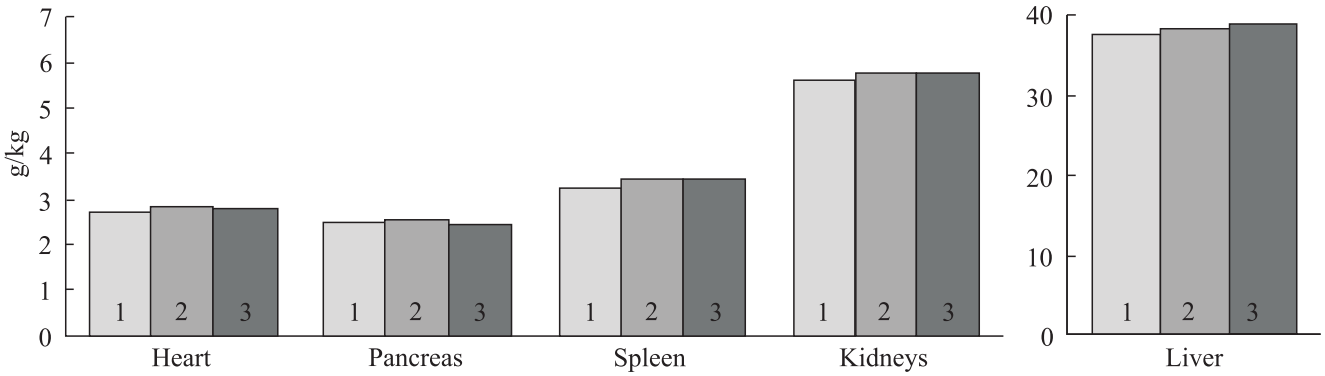

Fig. 3. The coefficients of the weight of internal organs of rats: 1 – group I, SR; 2 – group II, SR + 2.5 μg Ge; 3 – group III, SR + 2.5 mg Ge

as a higher dose does not change POL intensity in the blood of rats of group III, but has positive impact on the dynamics of their body weight. In particular, the body weight of animals of group II for 40 days of the testing period increased only by 27.7 % and amounted to 273.5 ± 4.85 g, whereas in the control group – by 32.6 % (284.8 ± 3.71 g) (Fig. 2). During the testing period the body weight of animals from group III amounted to 290.4 ± 2.78 g, and its surplus amounted to 34.9 %. Every ten days the body weight of rats from group III exceeded that of the control group by 6.9; 5.3 and 4.4 % during the first 30 days of the testing period, whereas in group II the indices of body weight on the 10th and 20th day were higher – by 4.0 and 3.2 % and on the 30th and 40th day – decreased down to 96.6 and 96.0 % respectively. In group III the body weight of rats on the 40th day exceeded the control by 2 %.

The decrease in the growth rate of animals of group II on the 30th and 40th days of feeding with Ge may indicate the slowdown of metabolic reactions after the period of adaptation of functional systems of the organism to the effect of low dose of Ge with the reduction in the metabolism intensity, thus – in growth and development. At the same time a high (2.5 mg/kg of body weight) dose kept its corrective biological impact for a longer period which ensures a higher growth of the organism in group III compared to control animals and experimental group II, and on the 30th and 40th days, although it was on a lower level compared to the first 20 days of the testing period.

Higher growth intensity of animals in groups II and III conditioned intergroup differences in the weight of internal organs and coefficients of their weight. In particular, the weight of the heart, liver, kidneys, spleen and pancreas for animals of group III exceeded these indices for control group rats by 5.5; 5.9; 7.5; 5.2 and 2.0 %, and for group II – by 1.8; 1.0; 2.5; 2.0 and 2.1 % (Table) 3).

However, higher intergroup differences in the weight of bodies of rats in group III leveled the differences in these indices between groups III, II and control group considerably, thus no reliable differences (against the actual weight of internal organs) were obtained (Fig. 3).

Thus, the physiological reaction of rats to the effect of low and high doses of GeNAC determines the changes

Table 3. The weight of internal organs of rats ($M \pm m$, $n = 5$), g

Organ	Group				
	I–control	II–2.5 μg Ge	% compared to control	III–2.5 mg Ge	% compared to control
Heart	0.78 ± 0.014	0.79 ± 0.027	101.8	0.82 ± 0.016	105.5
Liver	10.75 ± 0.23	10.86 ± 0.25	101.0	11.38 ± 0.211	105.9
Kidneys	1.59 ± 0.03	1.63 ± 0.04	102.5	1.71 ± 0.035*	107.5
Spleen	0.92 ± 0.02	0.93 ± 0.008	102.0	0.96 ± 0.010	105.2
Pancreas gland	0.70 ± 0.02	0.72 ± 0.006	102.1	0.72 ± 0.02	102.0

in the activity of haematopoietic, immune and oxidant systems, which affects the growth and development of the organism and internal organs of rats. A more expressed biological effect of Ge was revealed in the dose of 2.5 mg/kg of body weight, but the duration and frequency of administration of GeNAC requires experimental determination.

CONCLUSIONS

The physiological response of the immune, antioxidant and homeostasis functional systems of the organism of rats, their growth and development as regards the body weight and internal organs on feeding with low and high doses of Ge citrate stipulate unlike-directed changes in hematological and physiologically biochemical indices and is characterized by the following: 1) higher ($p \leq 0.05$) level of erythrocytes, EVF, leukocytes in the blood of animals of group III, its phagocytic activity and the content of ceruloplasmin – in group II; 2) lower level of indices of the organism reactivity – the content of average weight molecules in the blood of animals of groups II and III ($p \leq 0.05$), sialic acids, protein-bound hexoses as well as the intensity of peroxide oxidation of lipids by the content of TBA-active metabolites and lipid hydroperoxides in group II; 3) higher intensity of growth and development of the organisms of animals of group II by 3.2–4.0 % during the first 20 days, and group III – by 2.0–6.9 % during 40 days of feeding with Ge citrate as well as the weight of studied internal organs by 1.0–2.5 % and 2.3–7.5 % in groups II and III respectively.

Реакції фізіологічних систем організму щурів на випоювання низьких і високих доз «наноаквацитрату» германію

Р. С. Федорук, О. П. Долайчук,
І. І. Ковальчук, М. М. Цап

e-mail: cology@inenbiol.com.ua

Інститут біології тварин НААН України
Вул. В. Стуса, 38, Львів, Україна, 79034

Мета. Вивчити зміни стану окремих функціональних систем організму щурів у період випоювання різних доз «наноаквацитрату» германію. **Методи.** Використано автоматичний ветеринарний гематологічний аналізатор Orphee Mythic 18 Vet для визначення гематологічних показників. За допомогою колориметричних методів вивчали реактивність організму за вмістом молекул середньої маси, глікопротеїнів, моноцукрів їхніх вуглеводних компонентів у сироватці крові та за її фагоцитарною активністю, а також визначали гідропероксиди ліпідів і ТБК-активних метаболітів. Застосовано фізіологічні методи оцінки щодекадної динаміки змін маси тіла щурів, а після забою – маси серця, печінки, нирок, підшлункової залози, селезінки з визначенням їхніх масометричних коефіцієнтів. **Результати.** Встановлено міжгрупові відмінності в гематологічних показниках на тлі підвищення кількості еритроцитів, гемоглобіну, тромбоцитів і гематокриту поряд із зменшенням числа лейкоцитів та їхніх окремих форм у крові щурів дослідних груп, що вірогідно виражено в III групі. Аналогічну тенденцію відзначено для вмісту сіалових кислот і гексоз, зв'язаних з білками, у крові тварин III групи, ТБК-активних метаболітів – II, а молекул середньої маси – II і III груп. Реакція організму щурів залежно від інтенсивності їхнього росту підтверджує вираженіший біологічний вплив Ge в дозі 2,5 мг/кг маси тіла (із збереженням вищого рівня показників маси тіла) на 4,4−6,9 % і маси внутрішніх органів − на 5,2−7,5 % впродовж дослідного періоду. **Висновки.** Фізіологічна реакція імунної, антиоксидантної та гомеостазної функціональних систем організму щурів, їхнього росту і розвитку за масою тіла і внутрішніх органів на випоювання низької і високої доз цитрату Ge зумовлює неоднаково направлені зміни гематологічних і фізіолого-біохімічних показників та характеризується: 1) вищим ($p \leq 0,05$) рівнем еритроцитів, гематокриту, лейкоцитів у крові тварин III групи, її фагоцитарної активності та вмісту церулоплазміну – II групи; 2) нижчим рівнем показників реактивності організму – вмісту молекул середньої маси у крові тварин II і III груп ($p \leq 0,05$), сіалових кислот, гексоз, зв'язаних з білками, а також інтенсивності пероксидного окиснення ліпідів за вмістом ТБК-активних метаболітів і гідропероксидів ліпідів у II групі; 3) вищою інтенсивністю росту і розвитку організмів тварин II групи на 3,2−4,0 % у перші 20 діб, а III групи − на 2,0−6,9 % впродовж 40 діб випоювання цитрату Ge, а також маси досліджених внутрішніх органів на 1,0−2,5 і 2,3−7,5 % у II і III групах відповідно.

Ключові слова: «наноаквацитрат» германію, глікопротеїни, гематологічні показники, пероксидація ліпідів, щури, ріст і розвиток.

Реакции физиологических систем организма крыс на выпаивание низких и высоких доз «наноаквацитрата» германия

Р. С. Федорук, О. П. Долайчук,
И. И. Ковальчук, М. М. Цап

e-mail: ecology@inenbiol.com.ua

Институт биологии животных НААН Украины
Ул. В. Стуса, 38, Львов, Украина, 79034

Цель. Изучить изменение состояния отдельных функциональных систем организма крыс в период выпаивания разных доз «наноаквацитрата» германия. **Методы.** Использовали автоматический ветеринарный

гематологический анализатор Orphee Mythic 18 Vet для определения гематологических показателей. С помощью колориметрических методов изучали реактивность организма по содержанию молекул средней массы, гликопротеинов, моносахаров их углеводных компонентов в сыворотке крови и по ее фагоцитарной активности, а также определяли гидропероксиды липидов и ТБК-активных метаболитов. Применены физиологические методы оценки ежедекадной динамики изменений массы тела крыс, а после их забоя – массы сердца, печени, почек, поджелудочной железы, селезенки с вычислением их массометрических коэффициентов. **Результаты.** Установлены межгрупповые различия в гематологических показателях на фоне повышения количества эритроцитов, гемоглобина, тромбоцитов и гематокрита наряду с уменьшением числа лейкоцитов и их отдельных форм в крови крыс опытных групп, что достоверно выражено в III группе. Аналогичная тенденция отмечена для содержания сиаловых кислот и гексоз, связанных с белками, в крови животных III группы, ТБК-активных метаболитов – II, а молекул средней массы – II и III групп. Реакция организма крыс в зависимости от интенсивности их роста подтверждает более выраженное биологическое влияние Ge в дозе 2,5 мг/кг массы тела (с сохранением более высокого уровня показателей массы тела) в течение опытного периода на 4,4−6,9 %. **Выводы.** Физиологическая реакция иммунной, антиоксидантной и гомеостазной функциональных систем организма крыс, интенсивности их роста и развития по массе тела и внутренних органов на выпойку низкой и высокой доз цитрата Ge приводит к разнонаправленным изменениям гематологических и физиолого-биохимических показателей и характеризуется: 1) высшим ($p \leq 0,05$) уровнем эритроцитов, гематокрита, лейкоцитов в крови животных III группы, ее фагоцитарной активности и содержания церулоплазмина – II группы; 2) низким уровнем показателей реактивности организма – содержания молекул средней массы в крови животных II и III групп ($p \leq 0,05$), сиаловых кислот и гексоз, связанных с белками, а также интенсивности пероксидного окисления липидов по содержанию ТБК-активных метаболитов и гидропероксидов липидов во II группе; 3) более высокой интенсивностью роста и развития организмов животных II группы на 3,2−4,0 % в первые 20 сут, а III группы − на 2,0−6,9 % в течение 40 сут выпойки цитрата Ge, а также массы исследованных внутренних органов на 1,0−2,5 и 2,3−7,5 % во II и III группах соответственно.

Ключевые слова: «наноаквацитрат» германия, гликопротеины, гематологические показатели, пероксидное окисление липидов, крысы, рост и развитие.

REFERENCES

1. *Ishiwata Y, Yokochi S, Suzuki E, Michishita H, Tashita A, Asano K, Mitani T, Kurono M.* Effects of proxigermanium on interferon production and 2',5'-oligoadenylate synthetase activity in the lung of influenza virus-infected mice and in virus-infected human peripheral blood mononuclear cell cultures. *Arzneimittelforschung*. 1990;**40**(8):896–9.
2. *Kovalenko LV.* Evaluation of the stimulating effect of nanoaquachelates of germanium on the natural resistance of animals. *Naukovyy visnyk NUBiP Ukrayiny*. Ser. Veterinary medicine, quality and safety of animal products. 2012;**172**(1):203–9.
3. *Skal'nyy AV, Rudakov YA*. Bioelements in medicine. Moscow, Publishing House Onyx 21; Mir. 2004;272 p.
4. *Yl'yukhyn AB, Shkol'nykova LM, Seyfulyna YY, Batalova TP, Dyatlova NM.* Obtaining, structure and properties of the Germanium (IV) compound with nitrilotriacetic acid $Ge(Nta)(H_2O)(OH) \times 2H_2O$. *Russ J Coord Chem*. 1991;**17**(6):795−800.
5. *Sakhanda IV.* Preparations of germanium and their use in medicine. *Ukrainian Scientific Medical Youth Journal*. 2014;(4):83−6.
6. *Lin CH, Chen TJ, Hsieh YL, Jiang SJ, Chen SS.* Kinetics of germanium dioxide in rats. *Toxicology*. 1999; **132**(2−3):147−53.
7. *Pat.* of Ukraine No. 38391. 2009. Method for metal carboxylates obtaining "Nanotechnology of obtaining metal carboxylates". Kosinov MV, Kaplunenko VG. N a 200810939; appl. 08.09.2008; publ. 12.01.2009;Bull. N 1:5 p.
8. *Iskra RYa, Vlizlo VV, Fedoruk RS, Antonyak HL.* Chromium in animal nutrition. Kyiv, Ahrarna nauka. 2014;312 p.
9. *European* convention for the protection of vertebrate animals used for experimental and other scientific purposes: Council of Europe. Strasbourg. 1986;53 p.
10. *Vlizlo VV, Fedoruk RS, Ratych IB.* Laboratory methods of research in biology, and veterinary medicine. Lviv, Spolom. 2012;761 p.
11. *Kotsyumbas IYa.* Preclinical studies of veterinary medicines. Lviv, Triada plyus. 2006;360 p.
12. *Dolaychuk OP, Fedoruk RS, Kropyvka SJ.* Physiological reactivity and antioxidant defense system of the animal organism induced by Germanium, Chromium, and Selenium "nanoaquacitrates". *Agric Sci Pract*. 2015; **2**(2):50–5.
13. *Fedoruk RS, Dolaychuk OP.* Glycoproteins. Structure and function. *Naukovo-tekhnichnyy byuleten'*, Institute of Animal Biology NAAS. 2009;**10** (1–2):432–7.
14. *Lukevyts EYa, Har TK, Yhnatovych LM, Myronov VF.* The biological activity of Germanium compounds. Ryha, Zynatne. 1990;191 p.