ISSN 1814-6023 (Print)
ISSN 2524-2350 (Online)
UDC 616.36:616–099–092.4:612.441:661.722
https://doi.org/10.29235/1814-6023-2020-17-4-409-416 Received 06.04.2020

Valeria V. Lobanova, Frantishek I. Vismont

Belarusian State Medical University, Minsk, Republic of Belarus

INTERACTION OF LIVER ARGINASE AND L-ARGININE-NO SYSTEM IN THE PROCESSES OF DETOXIFICATION, LIPID PEROXIDATION AND THE FORMATION OF THE THYROID STATUS IN RATS WITH CHRONIC ETHANOL INTOXICATION

Abstract. A sufficient number of facts testifying the importance of liver arginase and nitrogen monoxide in the life processes in the normal and pathological conditions have been accumulated to date.

The aim of the study was to determine the significance of the relationship and interaction of liver arginase and L-arginine-NO system in the processes of detoxification, lipid peroxidation and the formation of the thyroid status in rats with chronic ethanol intoxication.

In rat experiments using the modern physiological, biochemical research methods and a pharmacological approach, it was found that chronic ethanol intoxication leads to a decrease in the liver arginase activity and the triiodothyronine concentration. At the same time, the increase in the level of "average molecules", NO_3^-/NO_2^-, the content of lipid peroxidation products in the plasma, as well as the increase in the blood toxicity degree, the activity of alanine amino transferase, aspartate amino transferase and the narcotic sleep duration were observed. Hyperthyroid rats demonstrated the increased liver arginase activity, the processes of detoxification, lipid peroxidation and body temperature while rats with the experimental hypothyroidism showed the opposite results. The liver arginase depression caused by the injection of N^{ω}-hydroxy-nor-L-arginine (Nor-NOHA), or L-valine into the body prevents the body temperature increase and the development of characteristic changes in the detoxification and lipid peroxidation processes acted upon by exogenous triiodothyronine. Under the conditions of the liver arginase inhibition by Nor-NOHA or L-valine, the ethanol action is accompanied by a more significant inhibition of the liver detoxification function and an increase of NO_3^-/NO_2^- levels in blood plasma. The preliminary injection of an N^G-nitro-L-arginine methyl ester inhibitor of NO-synthase into the animal's body weakens the toxic ethanol effect on the liver, as well as the development of characteristic changes in the liver arginase activity, in the processes of detoxification and lipid peroxidation in rats with chronic ethanol intoxication.

Apparently, the activity of liver arginase and L-arginine-NO system determines the severity of detoxification, lipid peroxidation processes and the formation of the thyroid status in the conditions of chronic alcoholization, which is important in the ethanol intoxication pathogenesis.

Keywords: chronic ethanol intoxication, detoxification, liver arginase, lipid peroxidation, L-arginine-NO system, rats

For citation: Lobanova V. V., Vismont F. I. Interaction of liver arginase and L-arginine-NO system in the processes of detoxification, lipid peroxidation and the formation of the thyroid status in rats with chronic ethanol intoxication. *Vestsi Natsyyanal'nai akademii navuk Belarusi. Seriya meditsinskikh navuk = Proceedings of the National Academy of Sciences of Belarus. Medical series*, 2020, vol. 17, no. 4, pp. 409–416. https://doi.org/10.29235/1814-6023-2020-17-4-409-416

В. В. Лобанова, Ф. И. Висмонт

Белорусский государственный медицинский университет, Минск, Республика Беларусь

ВЗАИМОДЕЙСТВИЕ АРГИНАЗЫ И L-АРГИНИН-NO СИСТЕМЫ ПЕЧЕНИ В ПРОЦЕССАХ ДЕТОКСИКАЦИИ, РАЗВИТИЯ ОКСИДАТИВНОГО СТРЕССА И ФОРМИРОВАНИЯ ТИРЕОИДНОГО СТАТУСА У КРЫС ПРИ ХРОНИЧЕСКОЙ ЭТАНОЛОВОЙ ИНТОКСИКАЦИИ

Аннотация. К настоящему времени накопилось достаточное количество фактов, свидетельствующих о том, что аргиназа печени и монооксид азота играют значительную роль в процессах жизнедеятельности в норме и при патологии.

Целью исследования было выяснение значимости взаимосвязи и взаимодействия аргиназы и L-аргинин-NO системы печени в процессах детоксикации, развития оксидативного стресса и формировании тиреоидного статуса у крыс при хронической этаноловой интоксикации.

В опытах на крысах с использованием современных физиологических, биохимических методов исследования и фармакологического подхода было установлено, что хроническая этаноловая интоксикация приводит к снижению активности аргиназы печени, концентрации трийодтиронина и к повышению уровня «средних молекул», NO_3^-/NO_2^-, содержания продуктов ПОЛ в плазме, степени токсичности крови, активности аланинаминотрансферразы и аспартатаминотрансферразы, продолжительности наркотического сна. У гипертиреоидных крыс повышается, а у крыс

с экспериментальным гипотиреозом снижается активность аргиназы печени, процессов детоксикации, ПОЛ и температура тела. Депрессия аргиназы печени, вызываемая введением в организм N^{ω}-гидрокси-нор-L-аргинином (Nor-NOHA), а также L-валина, препятствует повышению температуры тела и развитию характерных изменений процессов детоксикации и ПОЛ на действие экзогенного трийодтиронина. В условиях угнетения аргиназы печени как Nor-NOHA, так и L-валином действие этанола сопровождается более значимым угнетением детоксикационной функции печени и повышением содержания NO_3^-/NO_2^- в плазме крови. Предварительное введение в организм животным ингибитора NO-синтазы метилового эфира N^G-нитро-L-аргинина ослабляет токсический эффект этанола на печень, а также развитие характерных изменений активности аргиназы печени, процессов детоксикации и ПОЛ у крыс с хронической этаноловой интоксикацией.

По-видимому, от активности аргиназы и L-аргинин-NO системы печени зависит выраженность процессов детоксикации, ПОЛ и формирование тиреоидного статуса в условиях хронической алкоголизации, что играет значимую роль в патогенезе этаноловой интоксикации.

Ключевые слова: хроническая этаноловая интоксикация, детоксикация, аргиназа печени, перекисное окисление липидов, L-аргинин-NO система, крысы

Для цитирования: Лобанова, В. В. Взаимодействие аргиназы и L-аргинин-NO системы печени в процессах детоксикации, развития оксидативного стресса и формирования тиреоидного статуса у крыс при хронической этаноловой интоксикации / В. В. Лобанова, Ф. И. Висмонт // Вес. Нац. акад. навук Беларусі. Сер. мед. навук. – 2020. – Т. 17, № 4. – С. 409–416 (*на англ.*). https://doi.org/10.29235/1814-6023-2020-17-4-409-416

Introduction. Modern medicine faces the problem of the steady growth of alcohol pathology leading to a reduction in life expectancy and adversely affecting the state of health.

It is known that morbidity and mortality in case of regular consumption of alcohol is associated with the toxic effects of ethanol on the most important human organs, and especially on the liver [1, 2]. In the mechanisms of the protective reactions development in conditions accompanied by toxinemia, the activity of the liver detoxification function and the pituitary-thyroid system are important [3, 4].

Numerous experimental data indicate that toxic ethanol metabolites, activation of LPO processes, and the development of oxidative stress make a significant contribution to liver damage caused by ethanol [1, 5, 6].

Currently, a sufficient number of facts indicating the importance of liver arginase in the processes of detoxification and vital functions of the body in normal and pathological conditions has accumulated [7–9]. It has been shown that the activity of the metabolism of iodine-containing thyroid hormones [10], that play an important role in detoxification processes, depends on the liver functional state [3].

A number of researchers found that a change in the blood level of thyroid hormones is closely correlated with the production of nitrogen monoxide (NO) in the body [4] and arginase activity is important for its formation [11]. Considering that liver arginase activity limits the availability of L-arginine for inducible NO synthase [11, 12], it was reasoned that its activity will affect NO synthesis, that plays an important role in detoxification mechanisms, lipid peroxidation, and thermoregulation [13, 14]. However, the participation of liver arginase and L-arginine-NO system, the significance of their interaction in the regulation of its detoxification function, LPO processes and the formation of thyroid status in rats during chronic alcohol intoxication was not the subject of a special comprehensive study.

The aim of this study was to determine the significance of the relationship and interaction of liver arginase and L-arginine-NO system in the detoxification processes, the development of oxidative stress and the formation of thyroid status in rats with chronic ethanol intoxication.

Materials and research methods. The study was conducted on non-narcotic adult white male rats weighing 180–220 g.

The experiments were carried out at a strictly defined time (8–12 hours in the morning). The ration of rats consisted of the feed KK-92/PHC-5, the amount of which was determined by the Norms of feeding laboratory animals [15]. Drinking mode adhered to the ad libitum principle.

Due to the fact that in the literature there is evidence that animals have significant fluctuations in the level of a number of hormones and biogenic amines in the blood during the day, which are accompanied by changes in energy and plastic metabolism, the experiments were carried out at a strictly defined time (8–12 hours in the morning).

An experimental model of chronic ethanol intoxication was reproduced in rats by daily intragastric injection of 30 % ethanol solution to animals (based on 3.5 g of 92 % ethanol per kg of body weight) for 60 days. Acute toxic liver damage was caused by the intragastric injection of a solution of carbon

tetrachloride (CCl_4) prepared using olive oil at a ratio of 1:1 at a rate of 5 ml/kg to the animals. The activity of liver arginase was determined spectrophotometrically [16]. The production of nitric monoxide (NO) was evaluated by the total level of nitrates/nitrites (NO_3^-/NO_2) in blood plasma [17]. The detoxification function of the liver and the degree of endogenous intoxication were evaluated by the degree of blood toxicity (DBT), the duration of narcotic sleep (DNS), as well as by the concentration of "medium molecules" (MM) in the blood plasma. DNS (injection of hexenal at a dose of 100 mg/kg intraperitoneally) was evaluated by the time the animals were in the side position [18]. The method of acid-ethanol deposition [19] was used to determine the content of MM in the blood, and DBT was evaluated by the method proposed by O. A. Radkova et al. [20]. The severity of liver damage was estimated by the activity of aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) in the blood serum. The determination of the AsAT and AlAT activity in blood plasma was carried out using the colorimetric dinitrophenylhydrazine method.

The activity of lipid peroxidation in the blood and liver was evaluated by the content of such products as malondialdehyde (MDA), diene conjugates (DC), Schiff bases (SB). The concentration of MDA, DC, and SB was determined by the spectrophotometric method of M. Mihára, M. Uchiyama [21], V. A. Kostyuk [22] and B. L. Fletcher et al. [23], respectively.

Hypothyroidism in animals was reproduced using thyriostostatic mercazolilum (Ukrmedpreparaty, Ukraine), that was injected to rats daily intragastrically at a dose of 25 mg/kg on 1 % starch solution for 20 days. Experimental hyperthyroidism was reproduced using a synthetic preparation of triiodothyronine hydrochloride (Liothyronin, Berlin Chemie, Germany), that was injected intragastrally daily to animals at a dose of 30 μg/kg on 1 % starch solution for 20 days. The plasma level of triiodothyronine (T_3) and tetraiodothyronine (T_4) was determined by the radioimmunoassay using the test kits of OCP IBOKh NAS of Belarus. To determine the significance of liver arginase and NO in detoxification, thermoregulation and thyroid status formation, the arginase inhibitor N^{ω}-hydroxy-nor-L-arginine (nor-NOHA) (Bachem AG, Germany) and also L-valine (Carl Roth GmbH + Co.KG, Germany) and a non-selective inhibitor of NO synthase – methyl ester N^G-nitro-L-arginine (L-NAME) (ACROS ORGANICS, USA). Nor-NOHA at a dose of 10 mg/kg was injected to rats intraperitoneally daily for 7 days, and L-valine was injected intraperitoneally at a dose of 100 mg/kg 30 min before the start of the experiment. L-NAME at a dose of 25 mg/kg was injected to rats once intraperitoneally. Rectal temperature was measured with a TPEM-1 medical electrothermometer.

Blood and liver tissue for studies were taken for the minimum possible time after animal decapitation, that was carried out one hour after the last injection of ethanol (in the experimental group) or physiological solution (in the control group).

All experiments were carried out in accordance with the ethical standards for the handling of laboratory animals, as well as the requirements of the Directive of the European Ethics Committee 86/609 / EEC of 11.24.1986 [24] and the "European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes" dated March 18, 1986 and TCH 125-2008 "Good laboratory practice" approved by the resolution of the Ministry of Health of the Republic of Belarus No. 56 dated March 28, 2008 [25].

The received data were processed statistically using the software packages "Statsoft (USA) Statistica 8.0", "Microsoft Office Excell 2000", "Graph Pad Prism4". An analysis of the differences between the two independent groups by quantitative indicators, the distribution of which was not statistically significantly different from the normal, was carried out using the Student *t*-test in Welch's modification. Data for quantitative indicators are presented as arithmetic mean and standard error of the mean ($\overline{X} \pm S_x$), for qualitative indicators as percent. The differences between the experimental groups were considered significant at $p < 0.05$.

The results of the study. In experiments on rats, it was found that intragastric daily injection of 30 % aqueous ethanol solution to animals for 60 days leads to significant changes in arginase activity and liver detoxification function, body temperature, levels of lipid peroxidation products, NO_3^-/NO_2^-, tri- and tetraiodothyronine and plasma transaminase activity.

It was found that prolonged intragastric injection of ethanol leads to inhibition of the detoxification function of the liver, which was manifested by an increase in the level of MM in blood plasma –

by 38.5 % ($p < 0.05$, $n = 10$), DBT by 57.8 % ($p < 0$ 05, $n = 10$) and an increase in PNS by 24.5 % ($p < 0.05$, $n = 7$). The content of MM in blood plasma, DBT and DNS in the control group (with daily intragastric injection of a physiological solution for two months, $n = 10$) was 0.69 ± 0.012 g/l, 1.3 ± 0.11 units, respectively, and 27.8 ± 3.22 min. The liver arginase activity under the same conditions decreased by 54.7 % ($p < 0.05$, $n = 8$) and was 2.5 ± 0.27 μmol of urea/g of crude tissue per hour. The AsAT and AlAT activity as the most important indicators of the severity of liver damage, in the blood of alcoholized animals in comparison with the corresponding control increased by 196.3 % ($p < 0.05$, $n = 8$) and 488.5 % ($p < 0.05$, $n = 8$) and amounted to 1.77 ± 0.16 and 2.71 ± 0.13 μkat/l, respectively.

It was found that the effect of ethanol in the body of animals over 60 days is accompanied by an increase in blood plasma levels of DC, MDA and SB by 39.3 % ($p < 0.05$, $n = 7$), 58.5 % ($p < 0.05$, $n = 8$) and 50.8 % ($p < 0.05$, $n = 7$), respectively. The content of DC in the liver increased by 29.3 % ($p < 0.05$, $n = 7$), MDA by 36.5 % ($p < 0.05$, $n = 7$) and SB by 23.3 % ($p < 0.05$, $n = 7$). In rats of the control group (saline solution intragastrally daily for 60 days, $n = 8$), the content of DC, MDA, and SB in the blood plasma was 0.59 ± 0.051 D_{233}/ml, 0.71 ± 0.058 μmol/ml and 5.4 ± 0.52 IU/ml, and in the liver 14.5 ± 1.38 D_{233}/g tissue, 17.1 ± 0.71 μmol/g of tissue and 136.4 ± 13.5 U/g of tissue.

It was revealed that under conditions of chronic ethanol intoxication in animals, the concentration of NO_3^-/NO_2^- – end products of NO degradation in the blood plasma changes [11, 16]. Intragastric injection of ethanol after 60 days of alcoholization in rats ($n = 8$) resulted in an increase in plasma levels of NO_3^-/NO_2^- by 79.1 % ($p < 0.01$) and which amounted to 11.02 ± 1.34 μmol/l.

It was found that changes in thyroid status in rats occur as a result of chronic alcoholization. Prolonged (for 60 days) daily intragastric injection of 30 % ethanol solution led to a 58.8 % ($p < 0.05$, $n = 8$) decrease o fT_3 concentration in blood plasma. At the same time, T_4 concentration in comparison with the control group (daily intragastric injection of a physiological solution for 60 days) did not significantly change. The concentration of T_4 and T_3 in blood plasma in animals in the control group ($n = 7$) was 71.1 ± 11.04 and 1.7 ± 0.2 nMol/l, respectively.

It was found that 20 days after the daily intragastric injection of triiodothyronine hydrochloride (30 mg/kg), detoxification processes in animals are activated, liver arginase activity increases (by 41.0 %, $p < 0.05$, $n = 7$) and body temperature rises (by 0.7 °C, $p < 0.05$, $n = 8$). The DNS in rats under these conditions decreased by 27.2 % ($p < 0.05$, $n = 7$) and amounted to 20.9 ± 2.3 min. The content of MM in the blood plasma decreased by 23.5 % ($p < 0.05$, $n = 7$), and the degree of its toxicity decreased by 19.2 % ($p < 0.05$, $n = 7$). In this case, the plasma concentration of T_3 increased from 1.2 ± 0.1 to 1.9 ± 0.2 nMol/l (by 58.3 %, $p < 0.05$, $n = 8$) and T_4 decreased from 44, 7 ± 3.1 to 17.2 ± 2.0 nMol/l (by 61.5 %, $p < 0.05$, $n = 8$).

Depression of the functional activity of the thyroid gland with mercazolyl led to a decrease in liver arginase activity (by 25.6 %, $p < 0.05$, $n = 7$), inhibition of detoxification processes and a decrease in body temperature. So, before the injection of mercazolate, the rectal temperature in the rats of the experimental group ($n = 10$) was 37.3 ± 0.10 °C, and after 20 days of its use decreased by 0.9 °C ($p < 0.05$). The concentration of T_3 and T_4 in blood plasma in hypothyroid rats, compared with the control group (intragastric administration of 1 % starch solution for 20 days) decreased by 2.5 times ($p < 0.05$) and 3.2 times ($p < 0.05$) and amounted, respectively, to 0.54 ± 0.07 nMol/l ($n = 7$) and 16.4 ± 1.05 nMol/l ($n = 7$). The DNS in rats under these conditions increased by 28.2% ($p < 0.05$, $n = 7$) and amounted to 31.6 ± 2.85 min. The content of MM in the blood plasma of hypothyroid rats increased by 17.4 % ($p < 0.05$, $n = 7$), and DBT increased by 14.1 % ($p < 0.05$, $n = 6$).

It was revealed that in conditions of $CC1_4$ liver damage in rats, detoxification processes are inhibited, body temperature, liver arginase activity and plasma concentration of T_3 and T_4 decrease. So, 12 and 24 hours after the $CC1_4$ oil solution was injected into the stomach, the rectal temperature decreased, respectively, by 1.2 ± 0.12 °C ($p < 0.05$, $n = 12$) and by 1.7 ± 0.13 °C ($p < 0.05$, $n = 10$). The activity of liver arginase in rats ($n = 7$) under these conditions (in relation to animals in the control) decreased by 47.2 % ($p < 0.05$) and 61.8 % ($p < 0.05$), respectively, and the content NO_3^-/NO_2^- increased by 31.5 % ($p < 0.01$) and 58.4 % ($p < 0.01$), respectively. The activity of liver arginase in rats of the control groups (12 and 24 hours after intragastric injection of 1% starch solution) was 3.6 ± 0.30 ($n = 7$) and 3.8 ± 0.33 ($n = 7$) μmol of urea, respectively/g crude tissue per hour.

Acute toxic liver damage by CCl_4 led to an increase in plasma levels of MM and DBT. The concentration of MM after 12 and 24 hours from the time of seeding of animals CCl_4 increased by 28.2 % ($p < 0.05$, $n = 7$) and 39.1 % ($p < 0.05$, $n = 7$). Under these conditions, the DBT was higher in experimental rats compared to that in the control by 48.1 % ($p < 0.05$, $n = 6$) and 70.1 % ($p < 0.05$, $n = 7$). DNS in rats after 12 and 24 hours of CCl_4 injection increased compared to animals that received sunflower oil intragastricaly by 22.3 % ($p < 0.05$, $n = 8$) and 25.8 % ($p < 0.05$, $n = 9$), respectively. The duration of narcotic sleep in animals ($n = 7$) in the control group (12 and 24 hours after the injection of sunflower oil at a dose of 5.0 ml/kg into the stomach) was 22.8 ± 2.16 and 27.0 ± 1.73 min, respectively.

It was found that the action of CCl_4 in the animal organism is accompanied by the activation of lipid peroxidation processes in the blood and liver. So, 24 hours after the injection of CCl_4 oil solution into the stomach, the level of DC, MDA, and SB increased in blood plasma by 22.3 % ($p < 0.05$, $n = 7$), 32.2 % ($p < 0.05$, $n = 7$) and 81.4 % ($p < 0.05$, $n = 7$). In the liver, the content of DC increased by 20.5 % ($p < 0.05$, $n = 7$), MDA by 36.0 % ($p < 0.05$, $n = 7$), OR by 50.6 % ($p < 0.05$, $n = 7$). The action of CCl_4 in rats ($n = 8$) was accompanied by a decrease in the level of iodine-containing thyroid hormones in the blood plasma. So, 24 hours after the injection of hepatotropic poison to animals, a decrease in plasma levels of T_3 was observed – by 43.0 % ($p < 0.05$) and T_4 by 62.7 % ($p < 0.05$) compared with the control (intragastric injection of sunflower oil). The activity of AlAT and AsAT in blood after 12 and 24 hours after a single injection of CCl_4 oil solution (5.0 ml/kg) increased in experimental animals (compared with the corresponding control, intragastric injection of sunflower oil), respectively, by 518.5 % ($p < 0.05$) and 839.4 % ($p < 0.05$, $n = 6$), 136.7 % ($p < 0.05$, $n = 7$) and 204.5 % ($p < 0.05$, $n = 6$).

The results of the studies suggested that changes in body temperature and detoxification processes in rats under conditions of toxic liver damage as well as the depression of liver arginase and the L-arginine-NO system are mostly due to changes in the concentration of triiodothyronine in the blood plasma, which largely determines the activity of thermogenesis and detoxification processes.

It was found that daily intraperitoneal injection of the arginase inhibitor N^{ω}-hydroxy-nor-L-arginine (nor-NOHA) from BAChEM (Germany) to rats at a dose of 10 mg/kg [26] as well as a single intraperitoneal injection of the arginase inhibitor L-valine [27] at a dose of 100 mg/kg did not statistically significantly affect body temperature and led to a decrease in liver arginase activity by 70.8 and 78.6 % ($p < 0.05$, $n = 7$), respectively. In animals of the control group ($n = 7$), that received an intraperitoneal saline solution for a week, the activity of liver arginase was 5.7 ± 0.51 μM mol of urea/g of crude tissue per hour, respectively.

Under conditions of liver arginase depression by nor-NOHA, the action of ethanol is accompanied by a more significant inhibition of the liver detoxification function, an increase in plasma NO_3^-/NO_2^- level, the content of lipid peroxidation products in the blood and liver, and a decrease in body temperature. The body temperature in rats influenced by chronic ethanol intoxication decreased by 1.2 ± 0.16 ($p < 0.01$, $n = 12$), and under the conditions of action of nor-NOHA by 1.6 ± 0.13 °C ($p < 0, 05$, $n = 8$). The content of NO_3^-/NO_2^-, DC and MDA in blood plasma in rats with chronic alcohol intoxication ($n = 8$) that got nor-NOHA compared to the level in the control group of animals (alcoholization and intraperitoneal injection of saline solution, $n = 8$) was higher by 47.1 % ($p < 0.05$), 35.1 % ($p < 0.05$) and 29.8 % ($p < 0.05$), respectively.

Acute toxic liver damage, 12 and 24 hours after the intragastric injection of CCl_4 was accompanied by a more significant decrease in body temperature and a significant increase in DNS toxicity of plasma and the level of MM in it in animals ($n = 7$), that were injected intraperitoneally with L-valine (100 mg/kg) daily for 7 days. Thus, the body temperature in rats of the control group, that were preliminarily injected intraperitoneally with physiological saline solution during the week before the intragastric injection of CCl_4 oily solution, decreased by 1.2 °C after 12 and 24 hours from the moment of hepatotropic poison injection ($p < 0.05$, $n = 10$) and 1.5 °C ($p < 0.05$, $n = 8$), and in the experiment, under conditions of preliminary intraperitoneal injection of L-valine, 12 hours and a day after the introduction of CCl_4, decreased by 1.7 °C ($p < 0.05$, $n = 7$) and 2.0 °C ($p < 0.05$, $n = 7$), respectively.

It was revealed that the action of CCl_4 in rats under conditions of liver arginase depression by L-valine is accompanied not only by a more significant inhibition of liver detoxification function, but also by more pronounced changes in the activity of AlAT and AsAT in animal blood plasma. It was also found

that the action of CCl_4 in the body, under the conditions of preliminary administration of L-valine to animals during the week, does not cause a decrease in T_4 level and exacerbates a decrease in T_3 concentration in blood plasma.

It was found that the effect of CCl_4 in animals that previously received L-NAME was accompanied by a less pronounced change in liver detoxification function. So, 24 hours after the injection of CCl_4, in conditions of depression of NO synthase by L-NAME, the content in the blood plasma of SM was lower by 22.3 % ($p < 0.05$, $n = 8$), and its toxicity decreased by 17.6 % ($p < 0.05$, $n = 8$) compared with the corresponding control (action only CCl_4). PNS in rats that received CCl_4 under L-NAME exposure conditions decreased by 29.0 % ($p < 0.05$, $n = 10$) after 24 hours intragastric injection of hepatotropic poison. It was found that the infusion of CCl_4, 24 hours after injection, in rats (previously treated intraperitoneally with L-NAME) leads to a more significant decrease in plasma T_3 concentration (by 23.1 %, $p < 0.05$, $n = 7$) and to a less pronounced (compared with animals that were injected with saline intraperitoneally and CCl_4 solution intragastrically) increase in the activity of AlAT and AsAT in blood plasma – by 26.7 % ($p < 0.05$, $n = 8$) and 24.0 % ($p < 0.05$, $n = 7$). Therefore, there was reason to believe that the thyroid status of the organism and the activity of detoxification processes depend not only on functional state of the pituitary-thyroid gland system, but also on the activity of arginase and L-arginine-NO of the liver system.

To verify the validity of our assumption, it was of interest to find out how the body temperature and activity of detoxification processes on the action of exogenous T_3 will change under conditions of L-arginine-NO system depression in animals.

The experiments showed that preliminary (12 hours before the intragastric injection of T_3) intraperitoneal administration to rats ($n = 8$) of L-valine (100 mg/kg) prevents the increase in body temperature induced by daily injection of T_3 (30 μg/kg) for 20 days.

In a special series of studies, it was found that the injection of exogenous T_3 to rats ($n = 8$) under conditions of action of an NO synthase inhibitor in the body (L-NAME, 25 mg/kg, intraperitoneally 30 min before the injection of triiodothyronine hydrochloride) does not lead to activation of detoxification processes and increase in body temperature. In the control group of animals (received saline instead of L-NAME, $n = 8$), an increase in body temperature was observed upon injection of T_3. Thus, an intragastric injection of triiodothyronine hydrochloride (30 μg/kg) to rats for 20 days, 30 min before the T_3 injection, who received an intraperitoneal saline solution, led to an increase in the rectal temperature of 0.8 °C in animals ($p < 0.05$, $n = 8$), and under the action of L-NAME (25 mg/kg), the action of T_3 in animals ($n = 8$) did not cause significant changes in body temperature.

DNS (hexenal 100 mg/kg intraperitoneally) in rats of the experimental group that received T_3 for 20 days under conditions of inhibition of the activity of NO synthase L-NAME increased 12 hours after the last intragastric injection of the hormone by 28.7 % ($p < 0.05$, $n = 7$) compared with control animals. The duration of narcotic sleep in control rats (intragastric injection of T_3 at a dose of 30 μg/kg for 20 days and saline intraperitoneally 30 min before injection of the hormone) was 20.4 ± 2.51 min ($n = 7$).

Along with an increase in DNS, hyperthyroid rats that preliminarily got L-NAME also showed an increase of MM plasma level up to 22.7 % ($p < 0.05$, $n = 7$) compared with animals in the control group. In experimental rats compared with those in the control DBT was higher by 24.3 % ($p < 0.05$, $n = 6$).

Therefore, under the conditions of action of the NO synthase inhibitor L-NAME in the body, triiodothyronine does not exert its characteristic activating effect on the processes of detoxification and thermogenesis.

It was found that the action of ethanol in rats, under conditions of preliminary (30 min before intragastrical injection of ethanol to animals for 60 days) injection of L-NAME into animals, as compared with the control group of animals, leads to less pronounced inhibition of detoxification processes. PNS, the level of SM in blood plasma and STK in experimental rats influenced by chronic alcoholization compared with animals of the control group (intraperitoneal injection of saline solution and chronic alcoholization, $n = 8$) were lower by 27.1 % ($p < 0.05$, $n = 9$), 48.3 % ($p < 0.05$, $n = 8$) and 24.2 % ($p < 0.05$, $n = 8$), respectively. The AlAT and AsAT activity in blood plasma in rats influenced by chronic alcoholization under conditions of action of an NO synthase inhibitor in animals was lower by 37.5 % ($p < 0.05$, $n = 7$) and 48, respectively 8 % ($p < 0.05$, $n = 7$), and the content of NO_3^-/NO_2^- by 39.1 % ($p < 0.05$, $n = 7$).

It was revealed that chronic ethanol intoxication in rats (n = 9) that previously received L-NAME, compared with animals of the control group, leads to a less significant increase in DC levels, namely, a decrease of DC level in the liver by 39.2 % ($p < 0.05$), and in blood plasma by 28.6 % ($p < 0.05$). The concentration of MDA in the liver under these conditions decreased by 27.6 % ($p < 0.05$), in plasma by 30.3 % ($p < 0.05$). The level of OS decreased in the liver and in blood plasma, respectively, by 50.5 % ($p < 0.05$) and 36.7 % ($p < 0.05$).

The revealed features of changes in the liver detoxification function and lipid peroxidation processes in the blood and liver, as well as the level of NO_3^-/NO_2^- in the blood plasma during chronic alcohol intoxication both in conditions of depression of liver arginase and the activity of the L-arginine-NO system, suggested that the activity of liver arginase and L-arginine-NO system determine the severity of detoxification processes and oxidative stress in chronic alcohol intoxication.

Conclusion. Chronic ethanol intoxication in rats is accompanied by a decrease in body temperature, blood T_3 level, liver arginase activity, an increase in DNS and in the content of lipid peroxidation products, levels of NO_3^-/NO_2^-, MM, DBT, as well as the activity of AlAT and AsAT in blood plasma. Liver arginase and the L-arginine-NO system are involved in changes in the detoxification function of the liver, lipid peroxidation processes, the thyroid status of the body, and body temperature induced by chronic ethanol intoxication. The action of the NO-synthase blocker L-NAME in the body weakens, but the arginase inhibitor nor-NOHA promotes the development of characteristic changes in the detoxification function of the liver and body temperature in chronic alcohol intoxication.

Thus, the results of our studies suggest that the activity of liver arginase and L-arginine-NO system determines the severity of detoxification processes, oxidative stress and the formation of thyroid status in conditions of chronic alcoholization, which is important in the pathogenesis of ethanol intoxication.

Conflicts of interests. The authors declare no conflict of interests.

References

1. Buko V. U., Lukivskaya O. Ya., Khokha A. M. *Metabolic effects of alcohol intoxication*. Minsk, Belorusskaya nauka Publ., 2005. 208 p. (in Russian).

2. Lelevich S. V., Barkovskii E. V. Central and peripheral metabolic mechanisms of chronic alcohol intoxication. *Narkologiya* [Narcology], 2013, no. 7, pp. 50–56.

3. Vismont F. I., Artyushkevich S. A. On the role of Kupffer cells and hepatocytes in the mechanisms of implementation of triiodothyronine influence on the processes of detoxification and regulation of body temperature. *Meditsinskii zhurnal* [Medical journal], 2005, no. 3, pp. 45–47 (in Russian).

4. Fernandez V., Cornejo P., Tapia G., Videla L. A. Influence of hyperthyroidism on the activity of liver nitric oxide synthase in the rat. *Nitric Oxide*, 1997, vol. 1, no. 6, pp. 463–468. https://doi.org/10.1006/niox.1997.0149

5. Moncada C., Torres V., Varghese G., Albano E., Israel Y. Ethanol-derived immunoreactive species formed by free radical mechanisms. *Molecular Pharmacology*, 1994, vol. 46, no. 4, pp. 786–791.

6. Tapiv G., Pepper I., Smok G., Videla L. A. Kupffer cells function in thyroid hormone-induced liver oxidative stress in the rat. *Free Radical Research*, 1997, vol. 26, no. 3, pp. 267–279. https://doi.org/10.3109/10715769709097805

7. Vismont A. F., Lobanok L. M. The role of arginase in liver detoxification process and its participation in the mechanisms of regulation of body temperature with bacterial endotoxemia. *Doklady Natsional'noi akademii nauk Belarusi* [Doklady of the National Academy of Sciences of Belarus], 2011, vol. 55, no. 2, pp. 83–87 (in Russian).

8. Méndez J. D., De Haro H., Conejo V. A. Spermine inereases arginase activity in the liver after carbon tetrachloride-induced hepatic injury in Long-Evans rats. *Biomedicine and Pharmacotherapy*, 2006, vol. 6, no. 2, pp. 82–85. https://doi.org/10.1016/j.biopha.2005.09.003

9. Proskuryakova T. V., Gurtovenko V. M., Trapeznikova S. S., Navasardyants D. I. Effect of alcohol intoxication on the activity of ethanol oxidation enzymes and rat liver arginase. *Voprosy meditsinskoi khimii* [Problems of medical chemistry], 1983, vol. 29, no. 4, pp. 95–98 (in Russian).

10. Greg Kelly N. D. Peripheral metabolism of thyroid hormones: a review. *Alternativ Medical Review*, 2000, vol. 5, no. 4, pp. 306–333.

11. Scibior D., Czeczot H. Arginine – metabolism and functions in the human organism. *Postępy Higieny i Medycyny Doświadczalnej*, 2004, vol. 58, pp. 321–332.

12. Hallemeesch M. M., Lamers W. H., Deutz N. E. Reduced arginine avaliability and nitric oxide production. *Clinical Nutrition*, 2002, vol. 21, no. 4, pp. 273–279. https://doi.org/10.1054/clnu.2002.0571

13. Teylor B. S., Alarson L. Ch., Billiar T. R. Inducible nitric oxide synthase in the liver: regulation and function. *Biochemistry (Mosc.)*, 1998, vol. 63, no. 7, pp. 766–781.

14. Gerstberger R. Nitric oxide and body temperature control. *Physiology*, 1999, vol. 14, no. 2, pp. 30–36. https://doi.org/10.1152/physiologyonline.1999.14.1.30

15. *On the norms of feeding laboratory animals and producers: Order of the USSR Ministry of Health, March 10, 1966, No 163.* Avialable at: http:// http://www.libussr.ru/doc_ussr/usr_6382.htm (accessed 01.04.2012).

16. Geyer J. W., Dabich D. Rapid method for determination of arginase activity in tissue homogenates. *Analytical Biochemistry*, 1971, vol. 39, no. 2, pp. 412–417. https://doi.org/10.1016/0003-2697(71)90431-3

17. Moshage H., Kok B., Huizenga J. R., Jansen P. L. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clinical Chemistry*, 1995, vol. 41, no. 6, pp. 892–896. https://doi.org/10.1093/clinchem/41.6.892

18. Park D. V. *The biochemistry of foreign compounds*. Long Island City, Pergamon, 1968. 282 p.

19. Moin V. M., Nikolaychik V. V., Kirkovskiy V. V. The method for determining the group of substances of middle molecules in biological fluids. A. s. 1520445 SSSR, VRB F 01 no. 33/50. *Otkrytiya. Izobreteniya* [Discoveries. Inventions], 1987, no. 41. 415 p. (in Russian).

20. Rad'kova O. A., Boyarinov G. A., Balishina I. N. A method for determining the toxicity of biological fluids. A. s. 1146570 SSSR, MKI b Ol no. 1/28. *Otkrytiya. Izobreteniya* [Discoveries. Inventions], 1985, no. 11. 616 p. (in Russian).

21. Mihara M., Uchiyama T. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, 1978, vol. 86, no. 1, pp. 271–278. https://doi.org/10.1016/0003-2697(78)90342-1

22. Kostyuk V. A., Potapovich A. I., Lunets E. F. Spectrophotometric determination of diene conjugates. *Voprosy meditsinskoi khimii* [Problems of medical chemistry], 1984, no. 4, pp. 125–127 (in Russian).

23. Fletcher B. L., Dillard C. L., Tappel A. L. Measurement of fluorescent lipid peroxidation products in biological systems and tissues. *Analytical Biochemistry*, 1973, vol. 52, no. 1, pp. 1–9. https://doi.org/10.1016/0003-2697(73)90327-8

24. *Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes*. Avialable at: http://www.eur-lex.europa.eu (accessed 21.05.2012).

25. *Technical Code of Good Practice 125-2008.Good Laboratory Practice, approved by the Republic of Belarus Ministry of Health decree № 56 from 28.03.2008*. Minsk, 2008. 35 p. (in Russian).

26. Boucher J. L. Selective inhibitors and substrates for arginases and nitric oxide syntheses. Fundamental & Clinical Pharmacology, 2004, vol. 18, no. 1, pp. 5–15.

27. Lerzynski G., Suschek C. V., Kolb-Bachoten V. In hepatocytes the regulation of NOS-2 activity at physiological L-arginine levels suggests a close link to the urea cycle. *Nitric Oxide*, 2006, vol. 14, no. 4, pp. 300–308. https://doi.org/10.1016/j.niox.2005.11.009

Information about the authors

Valeria V. Lobanova – Assistant. Belarusian State Medical University (83, Dzerzhinski Ave., 220116, Minsk, Republic of Belarus). E-mail: patfiz@bsmu.by

Frantishek I. Vismont – Corresponding Member, D. Sc. (Med.), Professor, Head of the Department. Belarusian State Medical University (83, Dzerzhinski Ave., 220116, Minsk, Republic of Belarus). E-mail: patfiz@bsmu.by

Информация об авторах

Лобанова Валерия Валерьевна – ассистент. Белорусский государственный медицинский университет (пр. Дзержинского, 83, 220116, г. Минск, Республика Беларусь). E-mail: patfiz@bsmu.by

Висмонт Франтишек Иванович – член-корреспондент, д-р мед. наук, профессор, заведующий кафедрой. Белорусский государственный медицинский университет (пр. Дзержинского, 83, 220116, г. Минск, Республика Беларусь). E-mail: patfiz@bsmu.by