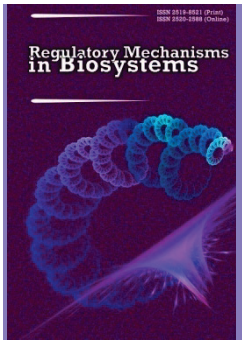

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Mitochondrial dysfunction of the inner membrane of hepatocytes in the development of glutamate-induced steatohepatosis and its correction

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Elucidation of the mechanisms of the development of liver steatosis, which are at the heart of the pathogenesis of nonalcoholic fatty liver disease (NAFLD), will allow the introduction of new effective treatment methods into medical practice, as well as the development of new measures for the correction of this disease and accompanying pathologies. The purpose of the research is to establish the enzymatic activity of the complexes of the electron transport chain of the mitochondrial membrane of rat hepatocytes and to evaluate the corrective effect of the multiprobiotic "Symbiter acidophilic" concentrated or nanocrystalline cerium dioxide on the formation of steatohepatosis induced by neonatal sodium glutamate administration. The experiments were carried out on 50 white non-linear male rats; the direction included the study of the mechanisms of the development of steatohepatosis in 4-month-old rats, which were administered monosodium glutamate in the neonatal period, and the study of the functional state of the liver in rats after the neonatal administration of monosodium glutamate against the background of periodic administration of a multiprobiotic or nanocrystalline dioxide cerium. It was established that neonatal administration of monosodium glutamate causes metabolic changes in 4-month-old rats, manifested in the disproportionate accumulation of fat with the development of visceral obesity without hyperphagia, dyslipidemia, and steatohepatosis. In 4-month-old rats, after neonatal administration of sodium glutamate, the development of steatohepatosis was accompanied by mitochondrial dysfunction, which was manifested by changes in the lipid composition of the inner membrane of hepatocyte mitochondria with an increase in oxidized products and a change in the enzymatic activity of all complexes of the respiratory chain. In rats injected with monosodium glutamate in the neonatal period, periodic use of the multiprobiotic "Symbiter acidophilic" concentrated or nanocrystalline cerium dioxide significantly restored the functional state of the liver, reduced the manifestations of oxidative stress and prevented the development of steatohepatosis, which indicates the antioxidant effect of these drugs and the possibility of their use for prevention of steatohepatosis.

Keywords: sodium glutamate; obesity; hepatic steatosis; multiprobiotic; nanocrystalline cerium dioxide.

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of clinical and morphological changes in the liver, represented by nonalcoholic fatty hepatosis, nonalcoholic steatohepatitis, fibrosis, liver cirrhosis, and hepatocellular carcinoma, which develop in patients who do not consume alcohol in hepatotoxic doses (Sheka et al., 2020). NAFLD is described as a hepatic manifestation of metabolic syndrome (Than et al., 2015). NAFLD is accompanied by the development of "mitochondrial dysfunction", one of the signs of which is oxidative stress, a violation of the functional activity of electron transport chain complexes (ETC or respiratory chain) together with a decrease in the level of ATP, DNA damage and changes in the lipid composition of the mitochondrial membrane. However, it remains unclear at what stage of NAFLD oxidative stress develops and whether it is a cause or a consequence of the development of this pathology. Currently, data on changes in the functioning of the ETC of hepatocyte mitochondria are pretty contradictory, as either a decrease in the activity of some complexes against the background of an increase in others or a reduction in the functional activity of the entire ETC can be observed (Pessayre, 2007; Paradies et al., 2014; Quines et al., 2016; Garcia et al., 2018). That is why it is relevant and appropriate to assess and compare changes in the activity of ETC complexes, which are characteristic signs of "mitochondrial dysfunction", under the conditions of the pathogenesis of NAFLD.

Even using the same model of obesity, such as knockout ob/ob mice (a leptin-deficient model of obesity that reduces energy expenditure and increases energy uptake), opposite results were produced regarding mitochondrial changes in the liver. Several studies have shown that mitochondrial bioenergetic activity in the liver of ob/ob mice is reduced (reduced activity of respiratory complexes, reduced respiration) (Garcia-Ruiz et al., 2007; Finocchietto et al., 2011), while other studies have shown that mitochondrial bioenergetic activity increases (Singh et al., 2009; Sharma et al., 2010). Interestingly, the work of Singh et al. (2009) showed that administering leptin to ob/ob mice, which led to a decrease in body weight and steatosis, caused a reduction in mitochondrial respiration in the liver. Lazarin et al. (2011) showed that H+-ATPase activity in the mitochondria of hepatocytes significantly increases in 4-month-old rats after neonatal administration of MSG.

The multidirectionality of the established data is caused by the polyetiological nature of steatohepatosis and indicates a rather complex and

multistage mechanism of pathology development (Aoun et al., 2012; Cardoso et al., 2013). The first stages of the development of the disease must be asymptomatic in most cases, which not only makes it difficult to establish the mechanisms but also prevents timely and effective treatment. Thus, most scientists emphasize that the development of steatohepatosis is accompanied by obesity, insulin resistance, diabetes, and hyperlipidemia (Attar et al., 2013; Gaggini et al., 2013). However, there are data that some patients may not be overweight and insulin resistant, but a relatively advanced form of NAFLD is diagnosed, accompanied by fibrosis and partial or complete loss of functional activity of the organ (Pan et al., 2002; Shi-Wen et al., 2008; Klass et al., 2009). That is why the research aimed to determine the functional changes of the mitochondrial membrane of rat hepatocytes under the conditions of the development of steatohepatosis.

An analysis of the literature on the role of intestinal microbiota in the development of obesity (Jia et al., 2018; Gerard et al., 2019) made it possible to choose two remedies as scientifically based corrective measures. The first is a probiotic preparation for normalizing the qualitative and quantitative composition of the intestinal microflora, multiprobiotic "Symbiter acidophilic" concentrated (NVP firm "O. D. Prolisok", Ukraine). The second is a drug with prebiotic activity, nanocrystalline cerium dioxide (NCD). The purpose of the study is to establish the enzymatic activity of the complexes of the electron transport chain of the mitochondrial membrane of rat hepatocytes and to evaluate the corrective effect of the multiprobiotic "Symbiter acidophilic" concentrated or nanocrystalline cerium dioxide on the formation of steatohepatosis induced by neonatal sodium glutamate administration.

Materials and methods

The experiments were carried out on 50 white non-linear male rats, which were kept in the vivariums of Danylo Halytsky Lviv National Medical University and Taras Shevchenko Kyiv National University in compliance with the rules of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) and approved by the First National Congress on Bioethics of Ukraine (Kyiv, 2001). The Commission on Bioethics of Danylo Halytsky Lviv National Medical University (protocol No. 5 dated June 22, 2020) and the Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (protocol No. 1 dated February 4, 2019) did not find moral ethical violations during experimental research. The animals were fed standard food "Purina rodent chow (fat – 20.6%, protein – 32.4%, carbohydrates – 47.0%) and water ad libitum.

The obesity model consisted of administering monosodium glutamate at a 4 mg/g dose dissolved in 8 μL/g of water by injection to rats in the neonatal period (Savcheniuk et al., 2014). Sodium glutamate was administered subcutaneously on the 2nd, 4th, 6th, 8th, and 10th days after birth. The total number of injections was 5. At the age of 1 month, the rats were randomly divided into three groups, the controls for which were intact rats of the appropriate age, which, after neonatal administration of monosodium glutamate, were injected with water in a volume of 0.25 mL/100 g (this was a 2-week course of administration for 3 months after one month of life). The second group of rats, after neonatal administration of monosodium glutamate, was injected with an aqueous solution of the multiprobiotic "Symbiter acidophilic" concentrated (140 mg/kg) in a volume of 0.25 mL/100 g (2-week course administration for three months after one month of life). The third group of rats, after neonatal administration of monosodium glutamate, was injected with nanocrystalline cerium dioxide (1 mg/kg), dissolved in water in a volume of 0.29 mL/100 g (2-week course administration for three months after one month of life). At the end of the experiment, at the age of 4 months, the rats were weighed and euthanized by cervical dislocation. Next, visceral adipose tissue (epidymal, perirenal and omentum fat) was prepared and weighed.

According to this method, administration of MSG in large doses to rats in the early neonatal period led to the destruction of the arcuate nuclei, as a result of which 16-week-old rats developed obesity and insulin resistance, as well as liver damage (Nakanishi et al., 2008). In rats injected with monosodium glutamate in the early neonatal period, body weight and food consumption registration occurred once a month, starting from 1 month after birth. The Lee Index determined obesity in each animal, the ratio of the cube root of body weight (g) to the rat's naso-anal length (cm). Rats with a Lee index value greater than 0.300 were classified as obese rats, and rats with a Lee index value close to or less than 0.300 were classified as normal rats (Bernardis et al., 1968). At the same time, hyperphagia did not develop, as the daily feed intake did not change. The data we obtained allow us to conclude that glutamate-induced obesity is not related to excessive caloric intake but results from a metabolic disorder.

To obtain functionally intact cells, the well-known non-enzymatic method of isolating the hepatocyte fraction of cells was modified (Hwang et al., 2001). Fragments of the inner membrane of mitochondria, which do not contain enzymes of the tricarboxylic acid cycle but include the entire set of carriers of the respiratory chain, are called submitochondrial fragments (particles) (SMF). The principle of obtaining submitted mitochondrial pieces (particles) of hepatocytes consists of the extraction of chopped tissue with a saline buffer solution, its destruction with the help of abrasive materials, and fractionation by stepwise centrifugation in Tris-sucrose buffer (Ardail et al., 1990).

Measurement of the enzymatic activity of ETC complexes in the inner membrane of mitochondria: the principle of the method for determining NAD·H-KoQ-oxidoreductase activity of the inner membrane of mitochondria consists in the restoration of cytochrome c NAD·H under the action of NAD·H-dehydrogenase (KF 1.6.99.3); determination of succinate-KoQ oxidoreductase activity of the inner membrane of mitochondria consists in the reduction of potassium ferricyanide ($K_3[Fe(CN)_6]$) to potassium ferrocyanide ($K_4[Fe(CN)_6]$) by succinate under the action of succinate oxidoreductase (KF 1.3.99.1); determination of KoQ-cytochrome c oxidoreductase activity of the inner mitochondrial membrane (complex b•c1, or complex III, KF 1.10.2.2) is based on the property of this complex to catalyze the oxidation of reduced ubiquinol by cytochrome c, which in turn is reduced (Schägger et al., 1995); determination of cytochrome oxidase activity of the inner membrane of mitochondria (KF 1.9.3.1) is based on the oxidation of cytochrome c by cytochrome oxidase; H+-ATPase activity of the inner membrane of mitochondria (KF 3.6.1.4) was determined as the amount of inorganic phosphorus, measured according to the Fiske-Subbarow method, calculated according to the calibration curve. The samples were photometered on an SF-46 spectrophotometer at a wavelength of 550 nm, and distilled water served as an optical control (Voieikova et al., 2016). The data were analyzed using Statistica 6.0 software pack (StatSoft Inc., USA). The data are presented in the diagrams as $x \pm SD$ (mean ± standard deviation). Differences between the values of the control and the experimental groups were determined using ANOVA, where the differences were considered statistically reliable at $P < 0.05$ (taking into account Bonferroni correction).

Results

As a result of the conducted research, a difference between the anthropometric parameters of rats of the control and experimental groups was revealed. Thus, in 16-week-old rats which were injected with monosodium glutamate in the early neonatal period, body weight and naso-anal length were, respectively, 9.4% ($P < 0.001$) and 23.7% ($P < 0.001$) smaller than similar indicators in rats of the control group (Table 1).

The decrease in naso-anal length in 4-month-old rats after neonatal administration of monosodium glutamate compared to a control group of rats results from a reduction of growth hormone secretion. Visually, 100% of rats injected with sodium glutamate in the neonatal period developed obesity, which was confirmed by the determination of the Lee index (0.364 ± 0.022 vs. 0.293 ± 0.031 in the control; $P < 0.05$).

Meanwhile, administration of monosodium glutamate in the neonatal period led to pronounced visceral obesity only, as the mass of visceral fat in these rats was 107.2% ($P < 0.001$) greater than that of control rats. In the second and third repetitions (the number of individuals in each sample – $n = 10$) of this series of studies, body weight did not change against the background of a statistically significant increase in visceral fat mass ($P < 0.001$), and in the fourth ($n = 7$) – there was also a decrease in body weight by 6.2% ($P < 0.05$) and the development of visceral obesity. At the same time, the increase in visceral fat mass was significantly more significant in rats of the second and third repetitions of this series of studies.

It was found that the decrease in body weight after neonatal sodium glutamate administration in the first and fourth repetitions was insignificant (9.4% and 6.2%). However, the rats of the first and fourth repetitions of this series of studies in the second and third months had a greater body weight than the control group rats. We deliberately did not combine the results of four repetitions into one sample, although the results of physiological and biochemical studies were unidirectional and close in value. We cannot reject seasonal influences on body weight. To establish changes in the eating behavior of rats with glutamate-induced obesity, feed consumption was determined in one-, two-, three-, and four-month-old rats. It was shown that the highest food consumption was in rats of both groups at the age of 4 months. Still, there was no statistically significant difference between the food consumption of the control group rats and those with glutamate-induced obesity. The data obtained allow us to conclude that glutamate-induced obesity is not related to excessive caloric intake but results from a metabolic disorder. Obesity was diagnosed by a high Lee index and was characterized by low body weight and naso-anal length compared to the control.

The studies revealed significant changes in the enzymatic activity of the electron transport chain (ETC) complexes in rat hepatocytes' mitochondria under glutamate-induced steatohepatosis conditions (Table 2).

In 4-month-old rats, after neonatal administration of sodium glutamate, NADH-KoQ oxidoreductase activity in hepatocyte mitochondria decreased by 56.0% ($P < 0.01$) compared to intact rats. Succinate-KoQ oxidoreductase activity in the mitochondria of rat hepatocytes after neonatal sodium glutamate administration decreased by 17.4% ($P < 0.05$) compared to the intact control. KoQ-cytochrome c oxidoreductase activity in the mitochondria of hepatocytes of 4-month-old rats after neonatal sodium glutamate administration decreased by 56.0% ($P < 0.01$) compared to intact animals of the same age. Cytochrome oxidase activity in the mitochondria of hepatocytes of 4-month-old rats decreased by 70.0% ($P < 0.001$) compared to intact animals after neonatal sodium glutamate administration. H^+-ATPase activity in the mitochondria of hepatocytes of 4-month-old rats decreased by 69.3% ($P < 0.001$) after neonatal sodium glutamate administration.

Table 1

Anthropometric parameters in 16-week-old rats after sodium glutamate administration in the early non-neonatal period (x ± SD, n = 15)

Indicators	Control (intact rats aged 16 weeks)	Experiment (rats aged 16 weeks after administration of monosodium glutamate in the early neonatal period)
Mass of rats, g	380.3 ± 26.0	344.4 ± 24.2
Naso-anal length, cm	25.3 ± 1.6	19.3 ± 1.4**
Body mass index, kg/m^2	5.94 ± 0.62	9.24 ± 1.11*
Lee's index, g$^{1/3}$/cm	0.293 ± 0.031	0.364 ± 0.022*
Mass of visceral fat, g	8.3 ± 1.0	17.2 ± 1.5***

Note: statistically reliable differences were considered compared with the control group: * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

Table 2

Enzymatic activity of ETC complexes under the conditions of glutamate-induced steatohepatosis in rat hepatocyte mitochondria (x ± SD, n = 10)

Indicator	Control	Glutamate-induced steatohepatosis
NADH-KoQ-oxidoreductase, μmol of potassium ferricyanide reduced/min x mg of protein	1.662 ± 0.083	0.731 ± 0.036***
Succinate - KoQ-oxidoreductase, μmol of potassium ferricyanide reduced/min x mg of protein	286.7 ± 14.3	236.8 ± 12.8*
KoQ-cytochrome c oxidoreductase, μmol cytochrome c reduced/min x mg protein	42.86 ± 2.14	18.86 ± 0.94***
Cytochrome oxidase, μmol of cytochrome c oxidized/min x mg of protein	114.3 ± 5.7	34.3 ± 1.7***
H+ - ATPase, μmol Pi / min x·mg of protein	312.4 ± 15.62	95.9 ± 4.8***

Note: see Table 1.

The presented results are evidence of a statistically significant decrease in the enzymatic activity of all ETC complexes and H+ - ATPase activity under the conditions of the development of glutamate-induced steatohepatosis, which indicates the growth of oxidative stress and the loss of regular functional activity of the mitochondrial membrane of hepatocytes. In this series of studies, four months after neonatal sodium glutamate administration, the weight of rats was 35.0% ($P < 0.05$) greater than the weight of rats in the control group (Table 3). At the same time, after neonatal monosodium glutamate administration, rats lagged in growth, and the naso-anal length was 18.1% ($P < 0.05$) less than the control. Lee's index confirmed the development of obesity in rats administered sodium glutamate in the neonatal period (Table 3). In rats of the control group, it was equal to 0.27 ± 0.03, and after 16 weeks after neonatal administration of sodium glutamate, it increased to 0.37 ± 0.03 ($P < 0.001$ compared to control). In 4-month-old rats, after neonatal administration of monosodium glutamate, the mass of visceral fat increased by 612.0% ($P < 0.001$) compared to the control (Table 3).

Periodic administration of the concentrated multi-probiotic "Symbiter acidophilus" to rats administered monosodium glutamate in the neonatal period prevented the development of obesity. The rats' body weight and naso-anal length decreased to the control level. The Lee index decreased significantly. Although the latter did not return to the control level, it fell within the limits (up to 0.30), which indicates the absence of obesity. In addition, periodic administration of the multi-probiotic to rats administered monosodium glutamate in the neonatal period led to significantly less visceral fat accumulation when the rats reached four months. Its mass decreased by 60.6% ($P < 0.001$) but did not reach the control level.

Eating behavior in rats after neonatal administration of MSG and in rats after neonatal administration of MSG against the intermittent administration of the multi-probiotic was the same as in the control group. Data on feed consumption evidence this. In all observation periods, there was no statistically significant difference between the data obtained on different groups of animals.

Therefore, the multi-probiotic "Symbiter acidophilic" concentrated under the conditions of periodic administration (2 weeks of administration, two weeks off) to rats that were administered monosodium glutamate in the neonatal period had a significant preventive effect on the development of obesity and steatohepatosis, which was confirmed by various methods.

Determination of the enzymatic activity of ETC complexes in the mitochondria of rat hepatocytes under the conditions of glutamate-induced steatohepatosis and its correction with the multiprobiotic "Symbiter acidophilic" concentrated showed changes in the functional activity of all ETC complexes (Table 4).

In 4-month-old rats, which were injected with monosodium glutamate in the neonatal period and which were periodically injected with a multi-probiotic until four months of age, NADH-KoQ oxidoreductase activity increased by 77.6% ($P < 0.05$) compared to rats with glutamate-induced steatohepatosis without correction. At the same time, it did not reach the level of the intact control and was smaller than it by 21.9% ($P < 0.05$). Periodic administration of a multi-probiotic to rats that were administered monosodium glutamate in the neonatal period did not affect the succinate-KoQ oxidoreductase activity of the inner membrane of hepatocyte mitochondria in 4-month-old rats compared to rats with glutamate-induced steatohepatosis without correction (Table 4). KoQ-cytochrome c oxidoreductase activity of the inner membrane of mitochondria of hepatocytes of rats after neonatal administration of sodium glutamate against the background of periodic administration of the multi-probiotic increased by 62.1% ($P < 0.01$) in comparison with the group of rats after neonatal administration of sodium glutamate. KoQ-cytochrome c oxidoreductase activity did not reach the level of the intact control.

Table 3
Anthropometric parameters in 16-week-old rats after administration of monosodium glutamate in the early neonatal period against the background of periodic administration of a multiprobiotic (x ± SD, n = 10)

Indicators	Control (rats aged 16 weeks)	Rats after neonatal sodium glutamate administration	Rats after neonatal administration of monosodium glutamate and against the background of periodic administration of the multi-probiotic
Mass of rats, g	250 ± 26^a	338 ± 26^c	288 ± 20^b
Naso-anal length, cm	23.2 ± 1.4^b	19.0 ± 1.2^a	22.0 ± 1.1^b
Lee's index, $g^{1/3}$/cm	0.27 ± 0.03^a	0.37 ± 0.03^c	0.30 ± 0.01^b
Mass of visceral fat, g	2.50 ± 0.40^a	17.82 ± 1.64^c	7.01 ± 0.81^b

Note: letters indicate significant differences between the subgroups within one line ($P < 0.05$) according to the Tukey test.

Table 4
Enzymatic activity of ETC complexes under conditions of glutamate-induced steatohepatosis in mitochondria of rat hepatocytes and correction with concentrated multi-probiotic "Symbiter acidophilic" (x ± SD, n = 10)

Indicator	Control (intact rats)	Rats after neonatal sodium glutamate administration	Rats after neonatal administration of monosodium glutamate + multi-probiotic
NADH-KoQ-oxidoreductase, µmol of potassium ferricyanide reduc./min * mg of protein	1.662 ± 0.083^c	0.731 ± 0.036^a	1.298 ± 0.039^b
Succinate-KoQ oxidoreductase, µmol of potassium ferricyanide reduc./min * mg of protein	287 ± 14^b	237 ± 13^a	244 ± 17^a
CoQ-cytochrome c-oxidoreductase, µmol of cytochrome c reduct./min * mg of protein	42.86 ± 2.14^c	18.86 ± 0.94^a	30.57 ± 1.03^b
Cytochrome oxidase, µmol of cytochrome c oxidn./min. * mg of protein	114.3 ± 5.7^c	34.3 ± 1.7^a	84.3 ± 3.2^b

Note: see Table 3.

Cytochrome oxidase activity of the inner membrane of the rat hepatocytes' mitochondria after neonatal monosodium glutamate administration was significantly affected by the periodic administration of the multiprobiotic. It increased by 145.8% ($P < 0.001$) compared to rats after neonatal sodium glutamate administration and remained 26.3% ($P < 0.01$) lower than this indicator in rats belonging to the intact control group.

H^+-ATPase activity of the inner membrane of mitochondria of hepatocytes of rats after neonatal administration of sodium glutamate against the background of periodic administration of the multi-probiotic led to its increase by 112.2% ($P < 0.001$) in comparison with the group of rats after neonatal administration of sodium glutamate. Compared with the intact control, H^+-ATPase activity of the inner membrane of mitochondria of rat hepatocytes was lower by 69.3% ($P < 0.01$).

Therefore, in 4-month-old rats after neonatal administration of monosodium glutamate against the background of periodic administration of the multiprobiotic, the increase in the enzymatic activity of ETC complexes indicates that oxidative stress was significantly weakened, which was manifested in a significantly lower severity of steatohepatosis.

In this series of studies, it was shown that the body weight of rats after neonatal sodium glutamate administration at four months was 35.2% ($P < 0.001$) greater remarkable than that of intact rats of the control group (Table 5). In 4-month-old rats injected with monosodium glutamate in the neonatal period and periodically throughout life with nanocrystalline cerium dioxide, the body weight was the same as in intact 4-month-old rats. Naso-anal length in 16-week-old rats after neonatal sodium glutamate administration decreased by 18.1% ($P < 0.001$) compared to intact controls. Periodic administration of nanocrystalline cerium dioxide to rats administered monosodium glutamate in the neonatal period did not affect the naso-anal length in 16-week-old rats. The Lee index, which was significantly greater than 0.30 and was 0.35 ± 0.03, confirmed the development of obesity in adult rats after neonatal sodium glutamate administration. Periodic administration of nanocrystalline cerium dioxide to rats after neonatal sodium glutamate administration returned the Lee index to control values. Visceral fat mass in 4-month-old rats increased by 612.0% ($P < 0.001$) after neonatal sodium glutamate administration. Periodic administration of nanocrystalline cerium dioxide to rats after neonatal administration of sodium glutamate reduced visceral fat mass by 60.6% ($P < 0.001$) compared to rats after neonatal administration of sodium glutamate. Compared to the intact control, the fat mass remained higher by 180.4% ($P < 0.001$, Table 5). Therefore, periodic administration of nanocrystalline cerium dioxide to rats after neonatal administration of monosodium glutamate prevented the development of obesity in adult rats.

Determination of the enzymatic activity of ETC complexes in the mitochondria of rat hepatocytes under the conditions of glutamate-induced steatohepatosis and its correction with nanocrystalline cerium dioxide showed changes in the functional activity of all ETC complexes (Table 6).

Table 5
Anthropometric indicators in 16-week-old rats after administration of monosodium glutamate in the early non-neonatal period and against the background of periodic administration of nanocrystalline cerium dioxide (x ± SD, n = 10)

Indicator	Control	Rats after neonatal sodium glutamate administration	Rats after neonatal administration of monosodium glutamate and intermittent administration of nanocrystalline cerium dioxide
Mass of rats, g	250 ± 16^b	338 ± 26^c	211 ± 40^a
Naso-anal length, cm	23.2 ± 1.4^b	19.0 ± 1.2^a	19.0 ± 1.1^a
Lee's index, $g^{1/3}$/cm	0.29 ± 0.03^a	0.35 ± 0.03^b	0.30 ± 0.01^a
Mass of visceral fat, g	2.50 ± 0.40^a	17.84 ± 1.62^c	7.01 ± 0.81^b

Note: see Table 3.

Table 6
Enzymatic activity of ETC complexes under conditions of glutamate-induced steatohepatosis in rat hepatocyte mitochondria and correction by nanocrystalline cerium dioxide (x ± SD, n = 10)

Indicator	Control	Glutamate-induced steatohepatosis	Glutamate-induced steatohepatosis + nanocrystalline cerium dioxide
NADH-KoQ-oxidoreductase, µmol of potassium ferricyanide reduced/min x mg of protein	1.662 ± 0.083^c	0.731 ± 0.036^a	0.997 ± 0.049^b
Succinate-KoQ-oxidoreductase, µmol of potassium ferricyanide reduced/min x mg of protein	286.7 ± 14.3^c	236.8 ± 12.8^b	189.5 ± 9.5^a
KoQ-cytochrome c oxidoreductase, µmol cytochrome c reduced/min x mg protein	42.86 ± 2.14^c	18.86 ± 0.94^a	25.71 ± 1.28^b
Cytochrome oxidase, µmol of cytochrome c oxidized/min. x mg of protein	114.3 ± 5.7^c	34.3 ± 1.7^a	75.7 ± 3.8^b
H^+-ATPase, µmol Pn/min x·mg of protein	312.4 ± 15.6^b	95.9 ± 4.8^a	91.9 ± 4.6^a

Note: see Table 3.

In 4-month-old rats, after neonatal administration of sodium glutamate, NADH-KoQ oxidoreductase activity in hepatocyte mitochondria decreased by 56.0% ($P < 0.01$) compared to intact rats. Compared with this group of rats, in 4-month-old rats after neonatal administration of sodium glutamate against the background of periodic administration of nanocrystalline cerium dioxide, NADH-KoQ oxidoreductase activity in hepatocyte mitochondria increased by 36.4% ($P < 0.05$). However, it did not reach the level of intact control.

Succinate-KoQ oxidoreductase activity in the mitochondria of rat hepatocytes after neonatal sodium glutamate administration decreased by 17.4% ($P < 0.05$) compared to the intact control. In rats, succinate-KoQ oxidoreductase activity in hepatocyte mitochondria continued to decline after neonatal administration of sodium glutamate against the background of periodic administration of nanocrystalline cerium dioxide. Compared to the intact control, it was smaller by 33.9% ($P < 0.01$).

KoQ-cytochrome c oxidoreductase activity in the mitochondria of rat hepatocytes after neonatal sodium glutamate administration decreased by 56.0% ($P < 0.01$) compared to intact animals. Compared with this group of rats, after neonatal administration of sodium glutamate against the background of periodic administration of nanocrystalline cerium dioxide, KoQ-cytochrome c oxidoreductase activity in the mitochondria of hepatocytes increased by 36.3% ($P < 0.01$).

Cytochrome oxidase activity in the mitochondria of hepatocytes of 4-month-old rats decreased by 70.0% ($P < 0.001$) compared to intact animals after neonatal sodium glutamate administration. On the contrary, cytochrome oxidase activity in the mitochondria of hepatocytes of 4-month-old rats after neonatal administration of sodium glutamate against the background of periodic administration of nanocrystalline cerium dioxide increased by 120.8% ($P < 0.001$).

H^+-ATPase activity in the mitochondria of hepatocytes of 4-month-old rats decreased by 69.3% ($P < 0.001$) after neonatal sodium glutamate administration. Compared with this group of rats, H+ - ATPase activity in the mitochondria of hepatocytes did not undergo statistically significant changes in rats after neonatal administration of sodium glutamate, which was periodically administered with nanocrystalline cerium dioxide.

Therefore, during the development of glutamate-induced obesity, a decrease in enzymatic activity was observed. Under periodically introduced nanocrystalline cerium dioxide, the functional activity of the mitochondrial membrane of hepatocytes was significantly restored. However, it did not return to the level of the intact control.

Discussion

Before summarizing the obtained results, it should be noted that a mandatory condition for the prevention and treatment of nonalcoholic steatohepatosis is a lifestyle change, that entails the elimination of risk factors for its development. These include overeating, eating food rich in readily available carbohydrates, obesity, a sedentary lifestyle, and type 2 diabetes. In the case of experimental diet-induced steatohepatosis, changing feed to a regular diet balanced over time gives positive results. The same applies to people (Aliusef et al., 2023; Sorout et al., 2023; Voroniuk, 2023). When it comes to obesity of hypothalamic genesis (in experimental conditions, this is steatohepatosis on the background of visceral obesity after neonatal administration of sodium glutamate), a lifestyle change will not fundamentally change the problem because it occurs under conditions of consumption of ordinary food.

The increased visceral fat content affects glucose and lipid metabolism, resulting in steatosis and liver inflammation. The results of numerous experimental and clinical studies of the pathogenesis of steatohepatosis of the liver obtained in recent years are contradictory. Some studies indicate that under the initial stages of fatty infiltration of hepatocytes, a significant increase in lipid oxidation processes can occur, especially concerning the functioning of transport processes through the mitochondrial membrane. Others emphasize the opposite effect of excessive intake of lipids and carbohydrates, namely the increase in the synthesis of triglycerides in the cytoplasm, which contributes to strengthening accumulation processes. Such ambiguity of experimental data is associated with relatively poor diagnosis of the early stages, which are asymptomatic in patients. It makes it difficult to establish a precise sequence of events accompanying the development of steatohepatosis. The only thing on which the opinions of most scientists agree is the central role of the development of "mitochondrial dysfunction" in the pathogenesis of this disease because violations of the normal functioning of mitochondria were observed in most experimental models and many patients with steatohepatosis (Sethi et al., 2007).

The so-called "mitochondrial dysfunction" develops in several stages, although today, these are hypothetical assumptions based on the analysis of already obtained experimental data. First, several studies point to the accumulation of oxidized quinone compounds due to impaired electron transfer between respiratory chain complexes. Typically, the functioning of the mitochondrial ETC is associated with forming of a certain amount of ROS. Still, the mitochondria's relatively efficient antioxidant system neutralizes these products' harmful effects. Under the conditions of "mitochondrial dysfunction", as established by several studies, a significant increase in the activity of ETC complexes can be observed, which is considered the first reaction to increased lipid oxidation under these conditions. Other studies show structural and functional disorders of the mitochondria of hepatocytes, according to which the activity of some ETC complexes increases against the background of a decrease in the activity of others. Both established variants of changes in the functional activity of the respiratory system, along with additional data, indicate the development of oxidative stress. The development of oxidative stress negatively affects not only the mitochondrion itself but also the hepatocyte as a whole, resulting in inflammation, apoptotic and necrotic phenomena that significantly reduce the functional activity of the organ (Begriche et al., 2006; Gusdon et al., 2014).

Literature data indicate that similar changes in the functioning of the ETC can occur under the development of steatohepatosis caused by the intake of a large amount of carbohydrates and lipids. In our case, the development of obesity is observed, in which there is an excessive accumulation of adipose tissue, which produces triglycerides in significant quantities, which enter the liver from the blood and cause the development of steatohepatosis. It is this factor that combines glutamate-induced steatohepatosis and diet-induced and suggests some similarity in the consequences of the development of this pathological process (Pessayre et al., 2007; Oliveira et al., 2011; Rolo et al., 2012; Gusdon et al., 2014; Paradies et al., 2014; Voieikova et al., 2016).

Our research showed that periodic administration of a multi-probiotic to rats starting at one month of age prevents the development of obesity in adult rats (Savcheniuk et al., 2014). This was confirmed by the significantly lower number of cases of obesity, the decrease in the Lee index, and the mass of visceral fat in rats after correction with the multi-probiotic "Symbiter acidophilic" concentrated compared to the group of animals after neonatal administration of monosodium glutamate without the use of the a multi-probiotic.

Other studies have shown that neonatal administration of MSG to rats produced similar changes in their anthropometric data. Still, there was an increase in subcutaneous fat mass, cell size, and epididymal adipose tissue mass (Braga, 2004) and a more significant increase in retroperitoneal fat mass compared to control rats (Cunha, 2010). We believe that the use of body mass index in rats is inappropriate. The obtained results confirm this. Body mass index in rats of the control group was equal to 5.94 ± 0.62, and in rats with glutamate-induced obesity – 9.24 ± 1.11. Therefore, we continued to work with the Lee index. According to the literature, the value of the Lee index in animals with average body weight is within 0.29–0.30 $g^{1/3}$/cm, and in animals with experimental obesity of various genesis, it ranges from 0.30 to 0.33 $g^{1/3}$/cm (Begriche et al., 2006; Akanya et al., 2015). In our experiments, the Lee index in control rats was 0.29 ± 0.03, and in rats after neonatal sodium glutamate administration – 0.36 ± 0.02 ($P < 0.001$). Therefore, administering monosodium glutamate to rats in the neonatal period led to visceral obesity at four months of age.

The most common cause of obesity is an imbalance between energy intake and expenditure. In gnotobiotic mice, it was established that intestinal microbiota as an environmental factor increases energy absorption from food, regulates metabolism, integrates central and peripheral regulatory signals of food consumption, and thus increases body weight. The main mechanisms by which gut microbiota contribute to host metabolism have been revealed in studies of germ-free mice, which were protected from the development of diet-induced obesity (DIO). One of the

critical mechanisms by which germ-free animals are protected from DIO is increased fasting-induced adipose factor (FIAF), also known as angiopoietin-like protein 4. FIAF is an inhibitor of lipoprotein lipase produced in the intestine, liver, and adipose tissue. Fabric contamination with the gut microbiota of germ-free mice inhibited FIAF expression in intestinal epithelial cells (Backhed et al., 2004). Thus, it leads to an increase in lipoprotein lipase activity, which increases the uptake of fatty acids by cells, the accumulation of triglycerides by adipocytes, and improved fat storage. Germ-free FIAF/- mice have obesity similar to their conventionally raised littermates. After contamination with gut microbiota, germ-free FIAF/- mice had a 57% increase in total body fat, similar to that observed in their wild-type littermates. Thus, germ-free FIAF-/- mice fed a high-fat, high-carbohydrate diet are unprotected against DIO. This suggests that FIAF is a mediator of microbial regulation of energy storage (Backhed et al., 2007).

Moreover, Backhed et al. (2007) also showed that the phosphorylated AMP-activated protein kinase (AMPK) levels in the muscles and liver were increased in germ-free mice. AMRK is a critical enzyme that controls the energy status of cells and activates vital enzymes of fatty acid oxidation in mitochondria, including acetyl CoA carboxylase and carnitine palmito transferase I, which indicates increased energy expenditure. The precise pathway through which the microbiota signals to the liver or skeletal muscle AMRK is unknown but appears independent of FIAF (Backhed et al., 2007).

Conclusion

In 4-month-old rats which were injected with monosodium glutamate in the neonatal period, visceral obesity developed without the manifestation of hyperphagia, characterized by dyslipidemia, impaired sensitivity of peripheral tissues to insulin, and the development of steatohepatosis. Periodic administration of concentrated or nanocrystalline cerium dioxide multi-probiotic "Symbiter acidophilic" to rats with glutamate-induced steatohepatosis significantly restored the morpho-functional state of the liver and prevented the development of steatohepatosis.

In 4-month-old rats, after neonatal administration of sodium glutamate, the development of steatohepatosis was accompanied by mitochondrial dysfunction, which was manifested by changes in the lipid composition of the inner membrane of hepatocyte mitochondria with an increase in oxidezed products and a change in the enzymatic activity of all complexes of the respiratory chain. There are not only structural changes in the membrane but also dysfunctional changes in the mitochondrion as a whole, which manifests itself in the fact that ROS is generated instead of ATP in the respiratory chain, and this causes the development of oxidative stress both in the mitochondrion and in the entire hepatocyte. In 4-month-old rats after neonatal administration of monosodium glutamate, which were periodically administered the multi-probiotic "Symbiter acidophilic" concentrated or nanocrystalline cerium dioxide, the lipid composition of the inner membrane of hepatocyte mitochondria and the enzymatic activity of all respiratory chain complexes were significantly restored.

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