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 development 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 cerium dioxide. 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 in 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 hepatitis, 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 polyeiological 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 steatohepatitis is accompanied by obesity, insulin resistance, diabetes, and hyperlipidemia (Attar et al., 2013; Gaggiotti 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; Sh-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 steatohepatitis.

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 effective effect of the multiprobiotic “Symbiter acidophilic” concentrated or nanocrystalline cerium dioxide on the formation of steatohepatitis 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 of the animals. 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 were analyzed using Statistica 6.0 software pack (StatSoft Inc., USA). The data were presented in the diagrams as 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 neonatal period, body weight and naso-anal length were, respectively, 9.4% (P < 0.001) and 23.7% (P < 0.001) smaller than the intact control rats (Bernardis et al., 1968). At the same time, hyperphagia did not develop, as the daily food intake did not change. The data 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 particles (pieces) 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 (Arzallu et al., 1990).

Measurement of the enzymatic activity of ETC complexes in the inner membrane of mitochondria: the principle of the method for determining NADH-KoQ-oxidoreductase activity of the inner membrane of mitochondria consists in the restoration of cytochrome c NADH under the action of NADH-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 (K3[Fe(CN)6]) to potassium ferrocyanide (K4[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 b1, 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 phosphorous, 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 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).

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, food 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.

In 4-month-old rats, after neonatal administration of sodium glutamate, body weight, and naso-anal length decreased to the control level. The Lee index decreased to the control level. The Lee index confirmed the development of obesity in rats administered sodium glutamate.

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

Table 1

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Control (rats aged 16 weeks)</th>
<th>Experiment (rats aged 16 weeks after administration of monosodium glutamate in the early neonatal period)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of rats, g</td>
<td>380.3 ± 26.0</td>
<td>344.4 ± 24.2</td>
</tr>
<tr>
<td>Naso-anal length, cm</td>
<td>25.3 ± 1.6</td>
<td>19.3 ± 1.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>5.94 ± 0.62</td>
<td>9.24 ± 1.11</td>
</tr>
<tr>
<td>Lee's index, g/cm²</td>
<td>0.293 ± 0.031</td>
<td>0.364 ± 0.022*</td>
</tr>
<tr>
<td>Mass of visceral fat, g</td>
<td>8.3 ± 1.0</td>
<td>172 ± 1.5***</td>
</tr>
</tbody>
</table>

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

Table 2

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Glutamate-induced steatohepatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-KoQ-oxidoreductase, μmol of potassium ferricyanide reduced/min x mg of protein</td>
<td>1.662 ± 0.083</td>
<td>0.731 ± 0.036***</td>
</tr>
</tbody>
</table>
| 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 oxidized/min x mg protein | 42.86 ± 2.14 | 18.86 ± 0.98*** |
| Cytochrome oxidase, μmol of cytochrome c oxidized/min x mg of protein | 114.3 ± 5.7 | 343 ± 1.7*** |
| H+-ATPase, μmol P_i / min x mg of protein | 312.4 ± 15.62 | 95.9 ± 4.8*** |

Note: see Table 1.

The presented results are evidenced 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 steatohepatitis, 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 acidophilus” 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 steatohepatitis, 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 steatohepatitis and its correction with the multi-probiotic “Symbiter acidophilus” 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 steatohepatitis without correction (Table 4). KoQ-cytochrome c oxidoreductase activity of the inner membrane of 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.
Anthropometric indicators in 16-week-old rats after administration of monosodium glutamate in the early neonatal period against the background of periodic administration of a multi-probiotic (x ± SD, n = 10)

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

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)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control (intact rats)</th>
<th>Rats after neonatal sodium glutamate administration</th>
<th>Rats after neonatal administration of monosodium glutamate + multi-probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-KoQ-oxido-reductase, μmol of potassium ferricyanide reduced/min * mg of protein</td>
<td>1.662 ± 0.083^a</td>
<td>0.731 ± 0.036^a</td>
<td>1.208 ± 0.039^b</td>
</tr>
<tr>
<td>Succinate-KoQ-oxido-reductase, μmol of potassium ferricyanide reduced/min * mg of protein</td>
<td>287 ± 14^b</td>
<td>237 ± 13^a</td>
<td>244 ± 17^a</td>
</tr>
<tr>
<td>CoQ-cytochrome c-oxido-reductase, μmol of cytochrome c reduced/min * mg of protein</td>
<td>42.86 ± 2.14^b</td>
<td>18.86 ± 0.94^a</td>
<td>30.57 ± 1.03^3</td>
</tr>
<tr>
<td>Cytochrome oxidase, μmol of cytochrome c oxidn./min. * mg of protein</td>
<td>114.3 ± 5.7^a</td>
<td>34.3 ± 1.7^a</td>
<td>84.3 ± 3.2^a</td>
</tr>
</tbody>
</table>

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 multi-probiotic. 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) compared to intact controls. Periodic administration of nanocrystalline cerium dioxide to rats administered monosodium glutamate in the neonatal period decreased oxidative stress, which 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 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.

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)

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

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)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Glutamate-induced steatohepatosis</th>
<th>Glutamate-induced steatohepatosis + nanocrystalline cerium dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-KoQ-oxido-reductase, μmol of potassium ferricyanide reduced/min * mg of protein</td>
<td>1.662 ± 0.083^a</td>
<td>0.731 ± 0.036^a</td>
<td>0.997 ± 0.049^b</td>
</tr>
<tr>
<td>Succinate-KoQ-oxido-reductase, μmol of potassium ferricyanide reduced/min * mg of protein</td>
<td>286.7 ± 14.3^b</td>
<td>236.8 ± 12.8^b</td>
<td>189.5 ± 9.5^b</td>
</tr>
<tr>
<td>KoQ-cytochrome c-oxido-reductase, μmol of cytochrome c reduced/min * mg of protein</td>
<td>42.86 ± 2.14^b</td>
<td>18.86 ± 0.94^a</td>
<td>25.71 ± 1.2^b</td>
</tr>
<tr>
<td>Cytochrome oxidase, μmol of cytochrome c oxidn./min. * mg of protein</td>
<td>114.3 ± 5.7^a</td>
<td>34.3 ± 1.7^a</td>
<td>75.7 ± 3.8^a</td>
</tr>
<tr>
<td>H⁺-ATPase, μmol Pa/min * mg of protein</td>
<td>312.4 ± 15.6^a</td>
<td>95.9 ± 4.8^a</td>
<td>91.9 ± 4.6^a</td>
</tr>
</tbody>
</table>

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 steatohepatitis 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 steatohepatitis, changing food 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 steatohepatitis on the background of visceral obesity after neonatal sodium glutamate administration). This is confirmed by the significant increase in subcutaneous fat mass, cell size, and epididymal adipose tissue mass compared to control rats (Cunha, 2010). We believe that the use of probiotics in 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 “Synbiotic acidophilic” concentrated compared to the group of animals after neonatal administration of monosodium glutamate without the use of 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/cm^3, and in animals with experimental obesity of various genesis, it ranges from 0.30 to 0.33 g/cm^3 (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 that regulates fat storage. Proceedings of the National Academy of Sciences, 101(44), 15718–15723.


leiden on glucose metabolism and mitochondrial function alterations induced by monosodium glutamate administration to rats. Amino Acids, 48(1), 137–148.


