INTRODUCTION.

Nonalcoholic fatty liver disease (NAFLD) or steatohepatitis has become more widespread in human population and it is often accompanied by pathological disorders of other organs and systems. The combination of obesity with the development of type 2 diabetes affects liver disorders of other organs and systems. The combination of obesity with the development of type 2 diabetes affects liver

PHOSPHOLIPID COMPOSITION IN THE INNER MITOCHONDRIAL MEMBRANE OF RAT HEPATOCYTES UNDER THE DEVELOPING OF DIFFERENT TYPES OF STEATOHEPATOSIS

Nonalcoholic fatty liver disease (NAFLD) or steatohepatitis has recently become widespread, but its pathogenesis has not been thoroughly understood for today. Most scientists have appropriated a central role in the mechanisms of its development to mitochondria and so-called "mitochondrial dysfunction," which is observed in most animal models and in most patients. The main characteristics of "mitochondrial dysfunction" are the disruption in complexes of electron transport chain (ETC, respiratory chain), the decrease of oxidative phosphorylation and the development of oxidative stress, as well as the changes in the level of β-oxidation and in the Krebs cycle functioning [5, 6, 16, 17].

Recent studies have shown that experimental animals have suffered from steatohepatitis of different degrees of severity under the conditions of various types of high-calorie diets (HCD) even without weight excess [1, 3, 14]. Also, there has been established the direct effect of excessive income of carbohydrates and lipids on the development of steatohepatosis [1, 3, 6].

The pathogenesis of steatohepatitis has not been clearly understood for today. But most scientists appropriate a central role in the mechanism of NAFLD development to mitochondria and to "mitochondrial dysfunction" which is observed in most animal models and in most patients. The main characteristics of "mitochondrial dysfunction" are the disruption in complexes of electron transport chain (ETC, respiratory chain), the decrease of oxidative phosphorylation and the development of oxidative stress, as well as the changes in the level of β-oxidation and in the Krebs cycle functioning [5, 6, 16, 17].

Recent studies have shown that experimental animals have suffered from steatohepatitis of different degrees of severity under the conditions of various types of high-calorie diets (HCD) even without weight excess [1, 3, 14]. Also, there has been established the direct effect of excessive income of carbohydrates and lipids on the phospholipid composition not only of plasma membrane,

References
but also on the composition of inner mitochondrial membrane in different diets [2, 14, 15]. L-monosodium glutamate (MSG) is one of the most popular nutritional supplements which causes steatohepatosis and associated obesity. It is also characterized by the development of "mitochondrial dysfunction", which is similar in certain parameters to diet-induced steatohepatosis [8, 9, 10]. But, changes in phospholipid composition of inner mitochondrial membrane under glutamate-induced steatohepatosis have not been studied.

The aim of this work was to determine phospholipid composition of inner mitochondrial membrane of rat hepatocytes under diet-induced and glutamate-induced steatohepatosis and to compare data about steatohepatosis developing of different types.

**MATERIALS AND METHODS.**

The experiments were performed on non-linear white male rats. Steatosis was performed in two ways: by keeping on high-caloric diet (HCD) # C 11024 and by neonatal subcutaneous injection of MSG. 20 rats of 180-200g were taken and divided into two groups before the start of the experiment. HCD # C 11024 (Research Diabetes, New Brunswick, NJ) consisted of a standard feed (47%), condensed milk (44%), corn oil (8%) and starch (1%). This diet induces the development of steatosis in mice and rats [14]. The rats of the first group were kept on a standard feed with free access to water. The rats of the second group were kept on diet # C 11024 with free access to water. Both groups of animals were kept for 20 weeks.

Earlier studies demonstrated the development of steatosis under glutamate-induced visceral obesity [8, 9]. Newborn rats were divided into two groups. The first group was injected subcutaneously by MSG in a dose of 4 mg/kg dissolved in isotonic saline in amount of 8 ml/g body weight on the 2, 4, 6, 8 and 10 days of life. The second group was injected subcutaneously with isotonic saline in amount of 8 ml/g weight in the same period. Rats of both groups were kept on a standard feed and with free access to water during 4 months of life.

A well-known non-enzymatic method for selection hepatocytes fractions (by Petrenko O. et al. [4]) was modified. Fractions of inner mitochondrial membrane were separated using gradual ultracentrifugation [13]. Extraction of lipids was performed by a method of Folch et al. with modifications [12]. Phospholipids separation was performed by thin layer chromatography [3].

**RESULTS AND DISCUSSION.**

Phospholipid compositions of inner mitochondrial membrane have been changed under diet-induced steatohepatosis (tab. 1). A small percentage increase of cardiolipin content since the 3-d week of keeping on a diet was established compared to the control group of animals that were kept on a standard diet. The content of cardiolipin in rats with glutamate-induced steatohepatosis also increased by 1.5 times (p<0.01) compared with the control group (tab. 2). It may indicate the similar mechanisms of developing steatohepatosis of different types which is accompanied by the attraction of inner mitochondrial membrane. One of the main functions of cardiolipin is the interaction with ETC complexes. Thus, cardiolipin provides a normal functional activity of the respiratory chain. Free radical oxidation affects the fatty acid composition of this phospholipid, thereby, reducing the affinity for ETC complexes. The result of such changes is the reduced activity of I, III and IV ETC complexes. Published data show that cardiolipin amount can rise only under conditions of oxidative stress, such as by increasing the number of oxidized cardiolipin forms [5, 7, 15, 17]. Its total content increases in the membrane, but, at the same time, non-oxidized cardiolipin amount decreases, which was observed in animals with diet-induced and glutamate-induced steatohepatosis [6, 7, 9, 17].

**Table 1. Amount of phospholipids in the inner mitochondrial membrane rat hepatocytes under conditions of diet-induced steatohepatosis (n=10, M±m)**

<table>
<thead>
<tr>
<th>Group</th>
<th>3 week</th>
<th>10 week</th>
<th>12 week</th>
<th>15 week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>Cardiolipin (%)</td>
<td>17.92±1.65</td>
<td>18.77±3.61</td>
<td>19.10±0.18</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylcholine (%)</td>
<td>34.53±2.43</td>
<td>33.09±3.01</td>
<td>33.71±0.84</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylethanolamine (%)</td>
<td>28.90±1.08</td>
<td>35.48±3.76</td>
<td>32.92±1.19</td>
</tr>
<tr>
<td><strong>HCD</strong></td>
<td>Cardiolipin (%)</td>
<td>22.01±1.85*</td>
<td>22.52±1.13*</td>
<td>26.37±0.16*</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylcholine (%)</td>
<td>32.32±4.42</td>
<td>29.73±2.22</td>
<td>25.83±1.33</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylethanolamine (%)</td>
<td>30.98±3.97</td>
<td>30.08±2.05</td>
<td>28.08±3.03</td>
</tr>
<tr>
<td><strong>Phosphatidylinositol and Phosphatidylserine (%)</strong></td>
<td>31.32±1.48</td>
<td>29.73±2.22</td>
<td>25.83±1.33</td>
<td>27.04±0.31</td>
</tr>
<tr>
<td><strong>Sphingomyelin (%)</strong></td>
<td>11.46±2.17</td>
<td>10.28±2.11*</td>
<td>12.95±1.37</td>
<td>9.60±0.45*</td>
</tr>
<tr>
<td><strong>Phosphatidylglycerol (%)</strong></td>
<td>3.09±0.029</td>
<td>4.15±0.03</td>
<td>4.86±0.08</td>
<td>2.50±0.64</td>
</tr>
<tr>
<td><strong>Cardiolipin (%)</strong></td>
<td>5.54±3.82**</td>
<td>3.02±4.17*</td>
<td>3.13±3.86**</td>
<td>1.15±2.09***</td>
</tr>
<tr>
<td><strong>Oxidized phosphatidylcholine (%)</strong></td>
<td>1.27±0.011</td>
<td>1.27±0.019</td>
<td>0.98±0.05</td>
<td>1.06±0.02</td>
</tr>
<tr>
<td><strong>Oxidized cardiolipin (%)</strong></td>
<td>2.46±0.98***</td>
<td>2.26±1.75**</td>
<td>1.98±0.17***</td>
<td>1.90±0.05**</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, *** p<0.001 – vs. control group

Table 1. Amount of phospholipids in the inner mitochondrial membrane rat hepatocytes under conditions of diet-induced steatohepatosis (n=10, M±m)
The amount of phosphatidylcholine in the inner mitochondrial membrane decreased a little under the conditions of modified diet, but only at the 3rd week. But we observed a significant increase in its oxidized form – oxidized phosphatidylcholine. Thus, the content of oxidized forms was increased by 1.9 times (p <0.01) since the 3rd week of keeping HCD. The greatest increase, by 2 times (p <0.001) was observed at the 12th week compared to animals that were kept on a standard diet. A similar pattern is observed in the content of phosphatidylethanolamine and in its oxidized form – oxidized phosphatidylethanolamine. A small reduction of phosphatidylethanolamine was observed only at the 3rd week without significant changes until the end of keeping animals on the HCD. On the contrary, oxidized phosphatidylethanolamine content increased a little starting from the 3rd week, with maximal increase by 1.7 times (p <0.01) at the 15th week compared to the control group. The content of phosphatidylcholine, phosphatidylethanolamine, and of their oxidized forms, has similarly been changed under the conditions of glutamate-induced steatohepatosis. Thus, the amount of phosphatidylcholine has decreased by 1.7 times (p <0.01), and the amount of phosphatidylethanolamine has decreased by 1.4 times (p <0.05). At the same time phosphatidylcholine content has increased by 4.5 times (p <0.001), and oxidized phosphatidylethanolamine content has increased by 9.2 times (p <0.001) under MSG neonatal injection. The obtained data have shown the similarity of contents changes of these two phospholipids and their oxidized form, as well as a bigger level of oxidative stress development under glutamate-induced steatohepatosis. Normally, a small amount of oxidized phosphatidylcholine and oxidized phosphatidylethanolamine is observed in the inner mitochondrial membrane, therefore, the increasing content of oxidized products is one of the results of oxidative stress developing [2, 5, 7, 16].

The development of diet-induced steatohepatosis has shown also changes in the amount of minor components of inner mitochondrial membrane. The percentage amount of phosphatidylinositol and phosphatidylserine, mixtures increased by 3.4 times (p <0.05) at the 3rd week of keeping on modified diet, but starting from the 10th week we have observed a slight decrease in this index relative to the control. The amount of sphingomyelin was changing similarly; at first, it increased by 2 times at the 3rd week (p <0.01) and then, it decreased by 1.4 times (p <0.05) at the 10th week; by 1.5 times (p <0.01) at the 12th week and 2.2 times (p <0.001) at the 15th weeks. The total amount of phosphatidylinositol and phosphatidylserine has not significantly changed in rats with glutamate-induced steatohepatosis; and amount of sphingomyelin has increased by 1.8 times (p <0.01) relative to controls. Having analyzed the published data and summarized the results, we assume that the changes in the content of minor phospholipids result from the breached ratio of total phospholipid content and they do not have functional effects on inner mitochondrial membrane [3]. The only additional effect of the complex changes of phospholipid composition is to reduce fluidity of inner mitochondrial membrane which makes additional destabilizing effect on a normal functional activity not only of membranes but of mitochondria in general.

CONCLUSIONS

Received data about the lipid composition of the inner mitochondrial membrane under the conditions of diet-induced and glutamate-induced steatohepatosis indicate the disturbance of its normal functional state. The increase of oxidized forms of major phospholipids including cardiolipin proves the development of oxidative stress under the conditions of both types of steatohepatosis. Also, it was found that the amount of oxidized forms of the major phospholipids in the membrane is significantly increased by the injection (administration) of MSG, which allows concluding about the greater level of the oxidative stress development under the conditions of glutamate-induced steatohepatosis.

### Table 2. Amount of phospholipids in the inner mitochondrial membrane rat hepatocytes under conditions of glutamate-induced steatohepatosis (n = 10, M ± m)

<table>
<thead>
<tr>
<th></th>
<th>Control group (%)</th>
<th>MSG group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine</td>
<td>33.48±2.81</td>
<td>19.6±0.88</td>
</tr>
<tr>
<td>Cardiolipin (%)</td>
<td>30.59±2.13</td>
<td>22.18±0.86</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (%)</td>
<td>12.37±1.02</td>
<td>11.42±0.75</td>
</tr>
<tr>
<td>Sphingomyelin (%)</td>
<td>3.21±0.51</td>
<td>5.84±1.10</td>
</tr>
<tr>
<td>Oxidized phosphatidylcholine (%)</td>
<td>1.13±0.12</td>
<td>10.39±0.87</td>
</tr>
<tr>
<td>Oxidized phosphatidylethanolamine (%)</td>
<td>0.36±0.029</td>
<td>1.98±0.17</td>
</tr>
</tbody>
</table>

* – p<0.05, ** – p<0.01, *** – p<0.001 – vs. control group
ФУНКЦИИ ФОСФОЛИПИДОВ В МИТОХОНДРИЯХ ЛИПИДОВ СТЕАТОГЕПАТОЗА

Введение

Стеатогепатоз - это состояние, при котором в печени происходит накопление жировых включений, приводящее к дисфункции органа. Фосфолипиды, в том числе и кардиолицин, играют важную роль в метаболизме жировых включений в печени.

Цель исследования

Изучение роли фосфолипидов, в том числе кардиолицина, в развитии стеатогепатоза.

Материалы и методы

Использовались животные модели стеатогепатоза, в том числе уточённые и винеальные грызуны. Были проведены исследования по определению содержания кардиолицина и других фосфолипидов в образцах печени.

Результаты

Показано, что уровень кардиолицина в печени животных с стеатогепатозом увеличивается, что свидетельствует о возрастании роли кардиолицина в обмене жировых включений.

Заключение

Фосфолипиды, включая кардиолицин, играют важную роль в развитии стеатогепатоза. Дальнейшие исследования могут помочь понять механизм развития и пути лечения этой патологии.

Ключевые слова: фосфолипиды, кардиолицин, стеатогепатоз.