ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Activity of Enzymes of Mitochondrial Electron Transport Chain of Rat Hepatocytes under Different Steatosis.

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ABSTRACT

The aim of this research was to determine the enzymatic activity of mitochondrial ETC complexes of rat hepatocytes under different types of steatosis. The study was carried out on white nonlinear male rats. Steatosis was performed in two ways: by keeping a high-caloric diet (HCD) # C11024 and by neonatal subcutaneous injection of L-monosodium glutamate (MSG). It was observed that enzymatic activity of mitochondrial ETC complexes of rat hepatocytes are different under the conditions of diet- and glutamate-induced steatosis development. Thus, the decrease of ATP synthesis and the development of oxidative stress under modified diet were observed. Also, similar decrease of H^+- ATPase activity but, at the same time, the decrease of enzymatic activity of all ETC complexes under conditions of glutamate-induced steatosis, which indicates a dysfunction of liver mitochondria was observed. The difference between the dates may be due to different mechanisms of pathological changes in hepatocytes under conditions of NAFLD induced by modified diet and MSG.

Keywords: steatosis, liver mitochondria, ETC complexes, high-caloric diet, L-monosodium glutamate

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ISSN: 0975-8585

INTRODUCTION

Steatosis or non-alcoholic fatty liver disease (NAFLD) is considered one of the most common forms of chronic liver disease for today. The disruption of diet, use of nutritional supplements, including L- monosodium glutamate (MSG), and physical inactivity are main factors that cause the steatosis development. Most patients with different phases of fatty liver suffer from abdominal type of obesity and/or from 2 type diabetes. Nevertheless, there are known some patients with NAFLD which are not overweight but with insulin resistance. The development of steatosis is accompanied with balloon degeneration, hepatic fibrosis and the increase of inflammatory cytokines in serum. The functional activity of the liver decreases as a result of mentioned above disorders [14, 15, 16].

Two key processes that accompany the development of different types of NAFLD, i.e., the excessive intake of triglycerides with the increased lipogenesis and the development of oxidative stress are discussed in different research papers. These disorders affect the state of liver and occur both together, and independently. The development of oxidative stress is closely connected with the changes in mitochondrial function which leads to mitochondrial dysfunction [8, 10, 14]. The main characteristics of mitochondrial failure are the changes in activity of electron transport chain (ETC, respiratory chain) complexes along with the decrease of ATP. The efficiency of ETC complexes functioning are performed by the activity of NADH: KoQ-oxidoreductase, succinate-KoQ-oxidoreductase, KoQ-cytochrome c oxidoreductase, and cytochromeoxidase as components of ETC complexes. The data on the role of ETC complexes in mitochondrial dysfunction under conditions of steatosis are not definitive [8, 9, 11, 13, 18]. Thus, the determination of ETC complexes activity is actual and expedient for assessing mitochondrial dysfunction progression under conditions of different types of steatosis.

The aim of this work is to determine the enzymatic activity of mitochondrial ETC complexes of rat hepatocytes under different types of steatosis.

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) # C11024 and by neonatal subcutaneous injection of MSG. We used 20 rats of 180-200 g and divided them 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 [12]. The rats of the first group were kept on a standard feed and 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 [17]. Newborn rats were divided in 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 rats 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. [1]) was modified. Fractions of inner mitochondrial membrane were separated using gradual ultracentrifugation [2]. NADH - KoQ-oxidoreductase activity was performed by the method of Pokrovskii O. et al. [3]. Succinate - KoQ-oxidoreductase, KoQ-cytochrome c-oxidoreductase, cytochrome oxidase activities were performed by standard methods [4, 5, 6]. H^+ - ATPase activity was performed by the method of Pikuleva O. et al. [7].

Statistical analysis of our research results was performed by common methods of variation statistics. Statistical analysis of parametric data was made by t-Student test for independent samples.

RESULTS AND DISCUSSION

Enzymatic activity of mitochondrial ETC complexes of rat hepatocytes changed after keeping male rats on HCD for 20 weeks (tab. 1). Thus, succinate - KoQ-oxidoreductase and H^+ - ATPase activity was lower by 1.2 ($p<0.05$) and 1.8 times ($p<0.01$) compared to the control group. NADH - KoQ-oxidoreductase, KoQ-cytochrome c oxidoreductase, cytochrome oxidase activity increased by 1.3 ($p<0.05$), 1.2 ($p<0.05$) and 1.2 ($p<0.05$) times

respectively. The increase of NADH - KoQ-oxidoreductase activity and the reduction of succinate - KoQ-oxidoreductase activity at this phase may be associated with the accumulation of electron-transporting intermediates like oxidized ubiquinone. Ubiquinone cannot transfer electron because of reduced activity of the II complex, and it gives away a molecule of oxygen forming a superoxide anion. This process stimulates the development of oxidative stress. Also, the synthesis of superoxide anion, instead of ATP, occurs during an electron transfer between III and IV complexes and under parallel reduction of H^+- ATPase activity. Similar changes in functioning ETC complexes happen under the development of steatosis and they are caused by the intake of a large number of carbohydrates and lipids; these facts reconcile with conditions modeled by us [8, 9, 10]. Considering the research data, we can assume the growth of oxidative stress due to the accumulation of intermediate electron-transporting compounds at such ETC complexes activity and under excessive income of lipids and carbohydrates.

The decrease of enzymatic activity of ETC complexes under the development of glutamate-induced steatosis have been established (tab. 2). Thus, NADH - KoQ-oxidoreductase activity decreases by 2.3 times ($p<0.01$), succinate - KoQ-oxidoreductase activity decreases by 1.1 times ($p<0.05$), KoQ-cytochrome c oxidoreductase activity decreases by 2 3 times ($p<0.01$), cytochrome oxidase activity decreases by 3.3 times ($p<0.001$) and H^+- ATPase activity decreases by 3.3 times ($p<0.001$). These data give reason to predict the decrease of ETC complexes activity and the reduction of ATP synthesis under glutamate-induced steatosis. Published data point out that triglycerides and cholesterol levels increase in serum under glutamate-induced obesity; and simple steatosis is also observed [11, 13, 17]. Our data indicate the decrease of ETC complexes enzymatic activity and of H^+- ATPase activity under these conditions. Mentioned above results are agreed with literature data about the state of respiratory chain and ATP level in mitochondria of intact hepatocytes under the development of glutamate-induced visceral obesity and NAFLD [18].

The development of steatosis, both diet- and glutamate-induced, caused a decrease H^+- ATPase activity which can be explained by to decrease of ATP synthesis in both cases. Also, in conditions of modeling NAFLD by different ways, there is a decline of succinate - KoQ-oxidoreductase activity. These data indicate an activity decrease of all ETC complexes and it reconciles with previously described characteristic features of mitochondrial dysfunction under glutamate-induced steatosis [18]. The character of ETC complexes' functional activity under excessive income of lipids and carbohydrates has been estimated. The changes in activity of ETC complexes indicate possible development of oxidative stress under these conditions. Thus, we observed differences in activity of ETC complexes under different models of steatosis, which could indicate possible different mechanism of NAFLD.

Table 1: Enzymatic activity of ETC complexes under conditions of diet-induced steatosis in mitochondria of rat hepatocytes

	Control group (n=10)	Group of diet-induced steatosis (n=10)
NADH - KoQ-oxidoreductase, mcmol potassium ferrocyanides restored/min x mg protein	1,579±0,078	1,990±0,099*
Succinate - KoQ-oxidoreductase, mcmol potassium ferrocyanides restored/min x mg protein	269,96±13,35	229,85±11,49*
KoQ-cytochrome c oxidoreductase, mcmol cytochrome c restored/min x mg protein	40,54±2,02	49,40±2,27*
Cytochrome oxidase, mcmol cytochrome c oxidation-reduction, /min x mg protein	113,07±5,65	136,81±6,84*
H^+- ATPase, mcmol phosphorus/min x mg protein	386,46±19,32	220,28±11,01**

* - $p<0,05$, ** - $p<0,01$ – vs. control group

Table 2: Enzymatic activity of ETC complexes under conditions of glutamate-induced steatosis in mitochondria of rat hepatocytes

	Control group (n=10)	Group of glutamate-induced steatosis (n=10)
NADH - KoQ-oxidoreductase, mcmol potassium ferrocyanides restored/min x mg protein	1,662±0,083	0,731±0,036**
Succinate - KoQ-oxidoreductase, mcmol potassium ferrocyanides restored/min x mg protein	286,71±14,33	236,79±12,84*
KoQ-cytochrome c oxidoreductase, mcmol cytochrome c restored/min x mg protein	42,86±2,14	18,86±0,94**
Cytochrome oxidase, mcmol cytochrome c oxidation-reduction /min x mg protein	114,3±5,71	34,29±1,71***
H^+- ATPase, mkmol phosphorus/min x mg protein	312,37±15,62	95,96±4,79***

* - p<0,05, ** - p<0,01, *** - p<0,001 - vs. control group

CONCLUSIONS

It was shown that enzymatic activity of mitochondrial ETC complexes of rat hepatocytes is different under conditions of diet- and glutamate-induced steatosis development. Thus, we have observed the decrease of ATP synthesis and the development of oxidative stress under modified diet. Also, we have observed a similar decrease of H^+- ATPase activity simultaneously with the decrease of enzymatic activity of all ETC complexes under conditions of glutamate-induced steatosis, which can indicate a dysfunction of liver mitochondria. The difference between the dates may be due to the different mechanism of pathological changes in hepatocytes in conditions of NAFLD induced by modified diet and MSG.

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