Cytochrome Oxidase Activity at The Hepatic Parenchyma Different Periods of Ischemia and Obstructive Jaundice

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Abstract- In this study, the activity of cytochrome oxidase was studied in different periods of the ischemic hepatic parenchyma and obstructive jaundice. It has been found that when liver damage occurs at different periods of ischemia and obstructive jaundice lead to inhibition of cytochrome oxidase.

Index Terms- liver, ischemia, hepatocellular damage, jaundice, animals, carbon tetrachloride

I. Introduction

Currently known methods for diagnosis of acute and chronic liver disease based on determining the activity of various enzymes in the blood and liver tissue, the rate of absorption of dyes and radioactive substances, the level of the metabolites synthesized by the organ [1,2,3,5,7]. However, none of these diagnostic tests do not provide a number of intact and functionally capable of liver parenchyma cells, and allows you to make only a qualitative picture of the pathological process in the body.

At the same time, such a diagnostic test is needed in Hepatology, it would allow to objectively represent the state of hepatic parenchyma in each case based approaches to selection of the treatment method and surgical volume, its validity and pathogenetic adequate postoperative period [4].

II. Material and Methods

Acute and chronic experiments on 200 rats weighing 160 - males made to solve the problem180 grams 46 dogs of various weights and sex.

By choosing as the main directions of the study of mitochondrial respiratory chain, responsible for liver cells and energy consuming 90% oxygen entering the cell, we examined the activity of cytochrome oxidase (cytochrome a a3 +). The latter has been studied by us in the presence of two substrates: cytochrome c -Natural electron donor and tetramethyl phenylenediamine (TMPD) -iskusstvennym electron donor. The need for such methodical reception due to the fact that reduced cytochrome C transmits electrons oligofermentnomu cytochrome oxidase complex which activates oxygen to form OH- via cytochrome a3. Recycled same TMPD oxidizes by cytochromes a + a3, localized on inner mitochondrial membrane (Jacob E / E., 1960).

To measure the activity in the presence of cytochrome oxidase and cytochrome C TMPD body piece taken in a Dounce homogenizer homogenate was prepared in a medium consisting of 0.25Msucrose 2x10 -4 EDTA, 0.01 M tris-HCl buffer (pH 7.6-7.8). The ratio of liver tissue and the medium was 1: 2 weight / volume. Polarographic analysis PL-7 (CSSR) was performed with a standard platinum electrode Clark closed.

The polarographic cell volume of 1.1 ml (t = 37°C) was added to the resulting homogenate is 1.4 mg protein calculating active volume of the cell. Then recorded the rate of oxygen consumption. Similar recordings were carried out with sequential addition of sodium ascorbate polarographic cell - analysis of final 2 mM TMPD and cytochrome C at a final concentration of 1 uM and 5 uM respectively. respiration rate expressed in nmol of O2 / min · mg protein. Predictive coefficient was calculated (PC) by the formula: PC = Cytochrome C -Askorbat Na / TMPD Na-Ascorbate

Digital material processed by the method of variation statistics.

III. Results and its discussion

Trying to use a cleaner model of damage to the liver parenchyma, we used ischemia.

Table №1
TMPD-activity and cytochrome C oxidase in liver ischemic animals

Period research	Ascorbate-dependent O2 consumption	TMPD oxidase activity	Cytochrome C oxidase activity	The ratio of cytochrome C / TMPD-oxidase activity
reference data	10.5 ± 0.15 N = 5	20.0 ± 1.5 N = 5	27.9 ± 3.0 N = 5	1.9 ± 0.05 N = 5
30 minutes ischemia % In the original data	10.5 ± 0.26 N = 5 P 0.05 100.0%	16.5 ± 0.45 N = 5 P 0.01 82,5%	28.6 ± 0.86 N = 5 P 0.05 102.5%	3.0 N = 5 P 0.01 157.9%
60 minutes ischemia % In the original data	12.0 ± 0.8 N = 5 P 0.05 114.3%	15.7 ± 0.9 N = 5 P 0.01 78,5%	33.8 ± 2.6 N = 5 P 0.05 121.0%	5.9 ± 0.06 N = 5 P 0.01 310.5%
120 minutes ischemia % In the original data	11.2 ± 0.36 N = 5 P 0.05 106.7%	15.9 ± 0.9 N = 5 P 0.01 79,5%	49.7 ± 6.3 N = 5 P 0.01 178.1%	.8,1 ± 0.07 N = 5 P 0.01 426.4%

As shown in Table №1, deprivation of oxygen to the liver in normothermia conditions almost did not change ascorbate-dependent oxygen consumption. TMPD-oxidase activity is reduced most significantly to the 60 minutes of ischemia (21.5%) and preserved substantially the 120 min study. Its level ranging from 60 minutes is increased by 1.2 times and continues to grow to 120 minutes of ischemia. After 180 minutes deprivation liver cytochrome C oxidase activity increases blood flow by 2.4 times (up to 67.3 ± 2.8 nmol of O2 / min-1 protein (r0,01, n = 5).

Interesting data were obtained in the group experiments with obstructive jaundice. Ligation of the common bile duct leading to the fact that after 7 days of increased activity by adding substrates of cytochrome: TMPD and cytochrome-C. If TMPD oxidase activity increased only 1.4 times, the cytochrome C oxidase activity increased significantly (3.1-fold). The increased level of the last saved when obstructive jaundice duration of 14 and 21 hours. During these periods, the study traced and higher activity TMPD oxidase. It is 2.4 times more as compared to the intact animals and 1.7 times the rats in which the obstructive jaundice duration of 7 days.

In this group of experiments, it was found that 7 days were critical period. And indeed there was a icterus paws and ears, decreased response to pain stimuli, ruffled fur, discharge from the nose ichor. At necropsy animals showed bleeding in the mesentery, the pleural cavity. The liver was their lemon-yellow color and a soft texture.

Table № 2
TMPD-activity and cytochrome C oxidase animal liver homogenates with obstructive jaundice

Period research	Ascorbate dependent O2 consumption	TMPD oxidase activity	Cytochrome C oxidase activity	Tsit. ratio C / TMPD-oxidase activity
Initial data	10.5 ± 0.15 N = 5	20.0 ± 1.5 N = 5	27.9 ± 3.0 N = 5	1.9 ± 0.05 N = 5
7 days obstructive jaundice % In the original data	20.8 ± 0.8 N = 5 P 0.01 198.1%	27.8 ± 0.9 N = 5 P 0.02 139.0%	87.9 ± 2.5 N = 5 P 0.01 315.0%	9.8 ± 0.3 N = 5 P 0.01 515.8%
14 days obstructive jaundice % In the original data	37.7 ± 0.9 N = 5 P 0.01 359.0%	47.5 ± 0.8 N = 5 P 0.01 237.5%	95.7 ± 0.6 N = 5 P 0.01 343.0%	5.9 + _006 N = 5 P 0.01 310.5%
21 days obstructive jaundice % In the original data	22.9 ± 0.7 N = 5 P 0.01 218.1%	32.1 ± 1.1 N = 5 P 0.01 160.5%	73.8 ± 4.5 N = 5 P 0.01 264.5%	5.5 ± 0.05 N = 5 P 0.01 289.5%

Visible intensive growth rate during the first 7 days of observation. And this turned out to be a critical period, after which 37 rats were killed 22 animals, ie, 59.4%. The rest of the animals survived and were derived from the experience of two months. They have a 14 and 21 days of study ratio was respectively 5.9 ± 0.06 and 5.5 ± 0.05 units (Table 4). At autopsy of these animals within a specified time occurred recanalization bile duct and the outflow of the restoration of the liver [5].

IV. Findings

1. liver lesions at different periods of ischemia and obstructive jaundice lead to the inhibition of cytochrome oxidase.

2. The ratio of cytochrome c / TMPD-oxidase activity is indicative of degradation of the inner mitochondrial membrane and disorders bioenergy hepatocytes.

3. Tier coefficient corresponds to a certain number of damaged (or preserved) liver parenchyma.

4. The use of the coefficient allows to predict disease outcome.

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