International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 18 No. 2 January 2020, pp. 146-150

Cytochrome Oxidase Activity at the Hepatic Parenchyma Toxic Lesions

[АКТИВНОСТЬ ЦИТОХРОМОКСИДАЗЫ ПРИ ТОКСИЧЕСКИХ ПОРАЖЕНИЯХ ПАРЕНХИМЫ ПЕЧЕНИ]

Akhmedov Mirhalil Dzhalilovich[1], Askarov Tohir Askarovich[2], Fayziev Yokub Nishanovich[3], Ashurmetov Ahmadjon Makhamadjonovich[4], Zhaffarov Hasan Mirzokhidovich[5] and Shounusov Sarvar Ikramovich[6]

[1]Tashkent Pediatric Medical Institute, assistant of the Department of General Surgery.

[2]Tashkent Pediatric Medical Institute, Doctor of Medical Sciences, Professor, Head of the Department of General Surgery.

[3]Tashkent Pediatric Medical Institute, candidate of Medical Sciences, Associate Professor of the Department of General Surgery.

[4]Tashkent Pediatric Medical Institute, Candidate of Medical Sciences, Associate Professor of the Department of General Surgery.

[5]Tashkent Pediatric Medical Institute, Assistant of the Department of General Surgery.

[6]Tashkent Pediatric Medical Institute, 3-year student of medical care.

Abstract - This study shows the activity of cytochrome oxidase in toxic lesions of the liver parenchyma. It has been found that when liver damage occurs at various periods of ischemia and obstructive jaundice, it leads to inhibition of cytochrome oxidase. A coefficient level was established corresponding to a certain amount of damaged (or intact) liver parenchyma.

Keywords - liver parenchyma, toxic damage, ischemia, animals, mitochondria.

Аннотация - в данном исследование показана активность цитохромоксидазы при токсических поражениях паренхимы печени. Выявлено, что при повреждении печени при различных сроках ишемии и обструкционной желтухе приводят к ингибированию цитохромоксидазы. Установлен уровень коэффициента соответствующий определённому количество повреждённой (или сохранной) паренхимы печени.

Ключевые слова - паренхима печени, токсическое поражение, ишемия, животные, митохондрия.

I. TOPICALITY

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

Corresponding Author: Akhmedov Mirhalil Dzhalilovich

validity and pathogenetic adequate postoperative period [4,6].

II. Material and Methods

To solve the problem, acute and chronic experiments were performed on 200 rats - men weighing 160–180 grams, 46 dogs of different weights and sexes.

Having chosen the study of the mitochondrial respiratory chain responsible for the energy of the liver cell and consuming 90% of the oxygen entering the cell as the main focus, we investigated the activity of cytochrome oxidase (cytochrome a + a3). The latter was studied by us in the presence of two substrates: cytochrome with –natural electron donor and tetramethyl-paraphenylenediamine (TMPD), an artificial electron donor. The need for such a method is due to the fact that the reduced cytochrome C transfers electrons to the cytochrome oxidase oligofermentation complex, which activates oxygen to form OH– through cytochrome a3. Restored TMPD is oxidized through cytochromes a + a3, localized on the 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) referred to coordinate the 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 ascorbatepolarographic 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 O_2 / 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

Toxic liver injury was induced in rats by administration of galactosamine and DL-carbon tetrachloride. Primer galactosamine in rats in a dose of 150 mg / 100 g resulted in death of all animals over 56 hours of observation. In this case, two-thirds of them die within the first two days. In animals, increases the clinical picture of acute liver failure. To this period of observation, they become adynamic, ruffled fur, animals do not drink water, and to painful stimulus response is reduced and slowed down. After 48 hours, seeding animal liver has a clay colored, loose, gives abundant scraping.

Table 1 TMPD-activity and cytochrome C oxidase (in nmol of O2 min-1 mg-1) in liver of animals inoculated galactosamine.

Theperiodofstudy	Ascorbate-dependentcon-sumptionAbout	TMPD oxidaseac-tivity	Cytochrome C oxidaseactivity	The ratio of cytochrome C / TMPD-oxidase activity
Initialdata	10.5 ± 0.15 N = 5	20.0 ± 1.5 N = 5	27,9 ± 3 N = 5	1.9 ± 0.05 N = 5
12 hours primer In% to the original.	17.1 ± 0.98 N = 5 P 0.005 162.8%	21.9 ± 1.1 N = 5 P 0.05 109.5%	41.8 ± 3.5 N = 5 P 0.01 149.8%	5.00 ± 0.005 N = 5 P 0.01 265.8%
18h primer In% to the original.	13.1 ± 0.6 N = 5 P 0.005 124.7%	17.0 ± 0.6 N = 5 P 0.05 85.0%	40.1 ± 1.16 N = 5 P 0.01 143.7%	6.94 ± 0.05 N = 5 P 0.01 365.3%
24 hours primer In% to the original.	13.5 ± 0.6 N = 5 P 0.05 128.6%	15.9 ± 0.6 N = 5 P 0.01 79,5%	36.8 ± 0.6 N = 5 P 0.01 131.9%	9.7 ± 0.15 N = 5 P 0.01 510%

48 hours of seeding In% to the original.	13.0 ± 0.4 N = 5 P 0.05 123.8%	15.1 ± 0.8 N = 5 P .01 75.5%	39.8 ± 4.4 N = 5 P 0.01 139.8%	13,2 ± 1 N = 5 P 0.01 694.8%

From the data presented in table 1, it follows that TMPD-oxidase activity by 12 o'clock observation practically retains the same level as in an intact liver. However, the activity of cytochrome-C-oxidase statistically significantly increased by 1.5 times. The ratio or ratio of these activities reaches 5.0– + 0.05 units. Such dynamics suggests that by 12 o'clock there are some changes in the respiratory chain of the mitochondrial electron transport. They can be associated with both terminal mitochondrial oxidase and the respiratory complex oxidized by TMPD. The latter assumption is more likely because the addition of exogenous cytochrome C, i.e. reconstruction of the respiratory chain leads to an increase in the circulation of the cycles in the mitochondrial electron transport complexes of the respiratory enzymes of mitochondria. The increase in TMPD-oxidase activity is a reflection of some changes occurring with endogenous cytochrome-C.

After 18 hours of the seed, DL-galactosamine, the activity of TMPD oxidase begins to fall statistically significantly. And in subsequent periods of observation (24 and 48 hours), such a decrease reaches the highest values (24.5%) compared with the control data. In the same periods of the study, an increased level of cytochrome C-oxidase is maintained. Moreover, compared with intact animals, this increase ranges from 31.9-43.7%.

Consequently, in rats with a duration galactosamine seed after 18 hours of study can be regarded as the threshold, although oxygen consumption increases by adding exogenous cytochrome-C in almost the same amount. TMPD oxidase activity is reduced not only due to changes occurring cytochrome-C respiratory chain, but also degradation of cytochrome a a3 +.

Table 2. Shows the results of experiments in animals poisoned by carbon tetrachloride.

Period research	Ascorbate-dependent O2 consumption	TMPD oxidaseactivity	Cytochrome-C oxidaseactivity	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
12 hours primer % In the original data	13.7 ± 0.36 N = 5 P 0.01 130.5%	21.9 ± 0.4 N = 5 P 0.05 109.5%	73,2 ± 3 N = 5 P 0.01 262.4% 0	7.2 ± 0.05 N = 5 P 0.01 378.9%
24 hour seed % In the original data	11.8 ± 0.4 N = 5 P 0.01 112.4%	14.8 ± 0.4 N = 5 P 0.01 74.0%	65.9 ± 2.0 N = 5 P 0.01 236.2%	10.9 ± 0.4 N = 5 P 0.01 573.7%

They point out that the 12 hours of study TMPD oxidase activity was not significantly changed. By 24 hours after seeding, and this drop is substantially 26% of the initial level. In this group of experiments, as in the experiments DL - galactosamine, there is a similar dynamic. Only in cases of carbon tetrachloride seed at a dose of 0.25 ml / 100 g body weight, the critical point is shifted to the 12 hours of the study.

Cytochrome C oxidase activity for this period increases 2.6 times and almost the same degree of increase (2.4 fold) is retained at 24 hours after seeding. Although we have unidirectional intensification of oxygen consumption by the addition of exogenous cytochrome-C in animals administered carbon tetrachloride and galactosamine, their intensity varies. The reason for such a difference needs further study, and possibly determined by the specificity of their action. This is apparently considerable importance is

that the point of application of the action galactosamine more hepatocyte plasma membranes are.

This leads to a more rapid degradation of the carrier of the respiratory chain, structured in the inner membrane of mitochondria, an exogenous cytochrome C and cytochrome possibly a + a3. May damage the mitochondrial membranes is most pronounced when the seed galactosamine. Although the conditions of defeat carbon tetrachloride may expect an increase in the role of free oxidation, i.e. reactions competing for oxygen.

In the later stages of seed CCL_4, when the pattern is clearly expressed structural defects of hepatocytes and, respectively, and its mitochondria, the activity of cytochrome C oxidase falls almost to the level that is present in animal, starting from 12-hour study period after seeding galactosamine. Thus, after 36 hours and 48 hours after administration of CCL_4 Activity of Cytochrome C oxidase is, respectively, 48.8 ± 1.3 and 46.7 ± 1.1 nM and 17.4 ± 0.25 ± 0.3 O_2 /min.-1 nmol mg-1 protein. Moreover, the level of the latter was not significantly different from that which is available in animals inoculated galactosamine, for 18-48 hours (p 0.005, n = 5).

Summarizing the data obtained by us in the process of modeling various pathological processes in the liver, it should be noted that the absolute values of the activities of terminal mitochondrial oxidase are subject to significant fluctuations. The scope of the latter is not controllable, since, like the activity of other enzymes, the complexes of the mitochondrial respiratory chain are subject to genetic, hormonal and other known types of control. Therefore, it would be a methodological mistake to recommend and use in practical hepatology cytochrome oxidase activity levels obtained by us, determined using TMPD and cytochrome C, in assessing the extent of damage to the liver parenchyma under various pathological conditions.

The assumption that the change in activity of cytochrome oxidase complex when used as a substrate TMPD damage caused by mitochondrial membranes, and changes that occur with the breathing carrier as cytochrome C. The causes of such changes may differ and relate to both the degradation of the enzyme itself, and with the solubilization and yield in the hepatocyte matrix of the inner mitochondrial membrane. Indeed, the addition of exogenous Cytochrome-C is largely restoring cytochrome oxidase activity. This fact was the basis for the assumption that the ratio of cytochrome C and TMPD oxidase activity will serve as an indicator of the degree of damage to mitochondrial membranes and degradation localized in the inner membrane of the respiratory carrier's subcellular structures.

Practically the same angle of inclination has a graphic curve coefficient values within the first 24 hours after seeding CCL_4. After this period, unlike galactosamine priming, the level ratio of cytochrome C / TMPD-oxidase activity begins to decline (tabl.2) and at 36 and 48 hours of study reaches respectively 7.1 and 0.04 ± 6.0 ± 0.04 units. It was found that at 24 hours in half of the animals die (23 out of 48 rats). The remaining animals in which the ratio was 6 units survived and continued to live for a long time after seeding CCL_4.

The data set above clearly indicate that those animals which have a higher value of the coefficient of 7-8 units die. Consequently, such values can diagnose severe forms of structural and functional disorders in the liver as a result of pathological process. And the specificity of this process does not matter. However, such an approach to diagnosing the state of the liver parenchyma did not give an idea of the quantitative characterization of the degree of damage to the liver parenchyma. To solve this problem, we adopted a methodical approach based on establishing in each case a correlation relationship between the coefficient and the number of viable hepatocytes. The last indicator was established by us in biopsied pieces of liver tissue, when one part of it was used to determine the ratio of cytochrome C and TMPD-oxidase activities, and the other for cell division. Viable hepatocytes were identified by staining with vital dye trypan blue and counted in a Goryaev's camera.

IV. Findings

Damage to the liver at various periods of toxic damage.

It leads to inhibition of cytochrome oxidase. The ratio of cytochrome C / TMPD-oxidase activities is an indicator of the destruction of the inner mitochondrial membrane and impaired bioenergy of hepatocytes. The level of the coefficient corresponds to a certain amount of damaged / or intact / liver parenchyma. Using the coefficient allows to predict the outcome of the disease.

References

[1] Dunaevsky O.A. Differential diagnosis of liver diseases. -M., Medicine 1985. -262 p.

[2] Podymova S.D. Liver Diseases: A Guide For Doctors.-M.:Medicine, 1998. -704 p.

[3] Masevich S.T., Ermolaeva L.G. Clinical, biochemical, morphological features of chronic hepatitis of various etiology //Therapeutic archive.- 2002.-№2.-P.35-37.

[4] Navruzbekov MS Estimation of the functional reserves of the liver and methods for predicting liver failure in liver operations // Abstract of dissertation of PhD. M., 2009.-34 p.

[5] SakhipovS.Zh. Violation of the functional state and microcirculation of the liver with mechanical jaundice. The dissertation of the candidate medical sciences - M., 1983.-162 p.

[6] Titov V.N. Biochemical methods for diagnosing liver pathology. // Therapeutic archive. - 1993, -№2.-P. 85-89.

[7] Khazanov A.I. Functional diagnosis of liver disease. -M., Medicine, 1988.-302p.