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Resume

The test of quantitative evaluation of the state of liver parenchyma has been developed on the basis of different models of hepatocelluiar injuries induced by effect of CCL4, DL-galactosamine, mechanical jaundice, ischemia in the laboratory animals. The test was based on determination of the coefficient obtained due to studying of the activity of cytochromoxidase during the, presense of two substrates cytochrome C and TMFD in the organ tissue homogenate.

Key words: DL-galactosamin, cytochromoxidasa, cytochrome C

МИТОХОНДРИАЛЬНЫЕ ФЕРМЕНТЫ В ОЦЕНКЕ ГЕПАТОЦЕЛЮЛЛЯРНЫХ ПОВРЕЖДЕНИЙ

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Резюме

На лабораторных животных с различными моделями гепатоцеллюлярных повреждений (затравка четыреххлористым углеродом - CCL4, DL-галактозамином, механическая желтуха, ишемия) разработан тест количественной оценки состояния паренхимы печени путем полярографических исследований. Тест основан на определении коэффициента получаемого в результате изучения в гомогенате ткани

органа активности цитохромоксидазы в присутствии двух субстратов: цитохрома С и ТМДФ (тетраметилпарафенилендиамина).

Ключевые слова: DL-галактозамином, гомогенат, цитохромоксидаза,

ГЕПАТОЦЕЛЮЛЛЯР ЖАРОҲАТЛАНИШДА МИТОХОНДРИАЛ ФЕРМЕНТЛАР АҲАМИЯТИ

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Резюме

Лаборатор ҳайвонларда эрталабки озиқланишида тўртхлор углерод-CCL4, DL-галактозамин билан турли гепато-целлюляр жароҳатланиш моделлари – механик сариқлик, ишемия ва гепато-биллиар соҳалар жароҳатида бемор жигари паренхимаси полярографик текширув орқали баҳолаш тести ишлаб чиқилди. Тест гомогенат тўқимада цитохромоксидаза активлиги коэффицентини аниқлаш цитохром С ва ТМДФ(тетраметилпарафенилендиамин) мавжудлигинида аниқлашга асосланган.

Калит сўзлар: DL-галактозамином, гомогенат цитохромоксидаза,

Relevance

When diagnosing the degree of hepatocellular damage in acute and chronic liver diseases, the use of a technique that allows to determine the number of intact and functionally capable cells of the liver parenchyma is relevant [1,2,3,6,8,9]. Since it makes it possible to objectively assess the state of the hepatic parenchyma in each specific case, to reasonably approach the choice of the method of treatment and the

volume of surgical intervention, its pathogenetic justification and adequate control of the course of the postoperative course [4,10].

Material and methods

To create an experimental model of hepatocellular insufficiency, experiments were carried out on 124 male Wistar rats. 5 series of experiments were carried out:

1. Control group.
2. After priming at a dose of 150 mg / 100 g of mass by intraperitoneal injection of DL-galactosamine.
3. After seeding by intraperitoneal injection of 0.25 ml / 100 g of CCL4 mass.
4. Model of obstructive jaundice after ligation of the common bile duct.
5. Model of normothermic ischemia.

The introduction of galactosamine leads to the development of acute liver failure, already after 24-48 hours with 100% death of animals after 56 hours. In contrast, with the introduction of CCL4, these changes were observed earlier (12-24 hours) after priming. 12-18-24 and 48 hours after the administration of galactosamine, 12 and 24 hours - CCL4; after 30, 60 and 120 minutes of normothermic ischemia; 7, 14 and 21 days after obturation - animals were killed by decapitation in a cold room. The liver was quickly removed, washed, and a homogenate was prepared in a medium consisting of 0.25 sucrose, 2x10-4 M EDTA (ethylenediaminetetraacetic acid); 0.01 M Tris-HCL buffer with pH 7.4 in the ratio of tissue and medium 1: 2. Polarographic analysis was performed with a standard closed-type Clarke platinum electrode on an LP-7 polarograph [7].

Into a 1.1 ml polarographic cuvette. the homogenate was introduced in turn at the rate of 1–2 mg. protein, sodium ascorbate at a final concentration of 2 mM, TMDF (tetramethylene paraphenylenediamine) -1 μM and cytochrome-C-1 μM. Respiratory rate was expressed in nmol O2 / minute. mg of protein. The prognostic coefficient (PC) was calculated by the formula:

PC = Cytochrome C - Ascorbate Na / TMPD - Ascorbate Na

The digital material was processed by the method of variation statistics.

Result and discussion

12 hours after priming with galactosamine, polarographic studies showed (Table 1), the rate of ascorbate-dependent consumption of O2 increased by 62.8% (P <0.05), further this indicator gradually decreases and by the end of the experiment this increase is only 23.8%. In contrast, the TMPD-oxidase activity of the liver homogenate gradually decreases (by 24.5% after 48 hours of inoculation). Cytochrome-C oxidase activity increased statistically significantly by 30-50% during the entire study period. In rats that received CCL4, we revealed the same dynamics of changes in the rate of consumption of 02 in various metabolic states. A distinctive feature of this model from galactosamine was a sharp activation of cytochrome C-oxidase (exceeding the values of intact rats by 162.4 and 136.2% after 12 and 24 hours from the priming). The model of normothermic ischemia did not have a significant effect on ascarbate, a dependent consumption of O2 by liver homogenates. TMPD-exidase activity was gradually inhibited, while cytochrome-C-oxidase activity gradually increased, exceeding the initial parameters by 78.1% by the 120th minute of ischemia. The creation of a model of obstructive jaundice contributed to an increase in ascorbate-dependent O2 consumption by 98.1; 259 and 118.1% after 7, 14 and 21 days, respectively, from the beginning of the experiment. In contrast to the above models, with this model, we did not observe inhibition of TMPD oxidase activity. Its values in all periods of the study reliably exceeded the standard parameters by 39; 137.5 and 60.5%, respectively. There was a sharp activation of cytochrome-C-oxidase activity, the values of which increased by 215; 243 and 164.5%, respectively.

Consequently, various models of liver necrosis significantly alter the rate of O2 consumption by liver homogenates. This is more pronounced in the model of obstructive jaundice. The observed inhibition of TMPD-oxidase activity of liver homogenates in chemical and ischemic damage, in our opinion, is associated with a

sharp disruption of the terminal portion of the respiratory chain, since according to the literature [8], there is a solubilization of cytochrome C localized on the outer side of the inner mitochondrial membrane, which limits transfer of electrons to the terminal section of the respiratory chain. Подтверждением нашего предложения является активация скорости потребления О2 при добавлении экзогенного цитохрома С. It should be said that the identified some of the distinctive features of the models used by us are associated with different points of their application.

With the models we used, there is a different lethality. Thus, the highest lethality was revealed when using the galactosamine model of liver damage, which, to a certain extent, does not coincide with the polarography data. To clarify the prognostic value of mitochondrial enzymes in assessing the degree of hepatocellular damage, we developed a prognostic coefficient. The choice of this formula was due to the localization of the used enzyme systems of the mitochondrial respiratory chain.

Analysis of this coefficient in animals inoculated with galactosamine showed a sharp increase up to 5.00 ± 0.05; 6.94 ± 0.005; 9.70 + 0.10; 13.20 ± 0.10 - 12, 18, 24 and 48 hours after priming, with a value of this indicator in intact rats of 1.90 ± 0.05. Moreover, its values coincided with the mortality during these periods of the study. This coefficient for CCL4 liver damage reached 7.20 ± 0.005 and 10.90 ± 0.04 (12, 24 hours after priming), and then decreased to 7.90 ± 0.005, but still exceeded the normative parameters. Moreover, the greatest mortality in this group was manifested after 24 hours, and later a decrease in mortality was noted.

Consequently, our proposed coefficient reflects the degree of damage to hepatocytes and coincides with the lethality of experimental animals. This is confirmed by the progressive increase in PC with the duration of ischemia. In contrast, PK values during obturation of the common bile duct were noted on the 7th day (increase to 9.80 ± 0530) 5 and then its gradual decrease, which coincided with the highest mortality during these periods. In our opinion, a decrease in

mortality at a later date is associated with recanalization of the common bile duct [5].

Table 1.

The rate of oxygen consumption (in nmol O2 min-1 mg-1) of the liver of experimental animals in various metabolic states

Study period	Ascorbate dependent	TMPD-oxidase	Cytochrome C-oxidase
Control	10,50±0,15	20,00±1,50	27,90±3,00
DL-galactosamine, after (hour): 12	17,10±0,98*	21,90±1,10	41,80±3,50*
18	13,10±0,60*	17,00±0,60	40,01±1,16*
24	13,50±0,60*	15,90±0,60*	36,82±0,60*
48	13,00±0,40*	15,10±0,80*	39,01±4,43*
CCL4, after 12 hours	13,73±0,37*	21,90±0,41	73,20±3,03*
24	11,82±0,41	14,85±0,44	65,90±2,02*
Ischemia, after (min.): 30	10,54±0,26	16,50±0,45*	28,60±0,86
60	12,01±0,80	15,72±0,91*	33,82±2,61
120	11,24±0,36	15,93±0,90*	49,72±6,32*
Obturation, after (days) 7	20,81±0,80*	27,82±0,90*	87,93±2,50*
14	87,93±2,50*	7,53±0,81*	95,71±0,61*
21	37,72±0,91*	32,11±1,13*	73,85±4,52*

*Note: * - the differences between the indicators of the control and experimental groups are significant (P <0.05)*

Conclusions

1. In rats with chemical and ischemic liver damage, a decrease in TMPD-oxidase activity of hepatocytes is observed due to the solubilization of

cytochrome C, since the addition of exogenous cytochrome C significantly activates the transfer of electrons along the respiratory chain.

2. In contrast to them, on the model of obstructive jaundice, ascorbate-dependent consumption of O2, TM PD- and cytochrome C-oxidase activity of hepatocytes is activated.
3. PC characterizes the degree of liver damage and coincides with the lethality. If with galactosamine and ischemic lesions of the liver, it progressively increases, then with CCL4 and the obstructive model it first sharply increases and gradually decreases further, which coincides with the lethality of animals in these groups.

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