ASSESSMENT OF THE MORPHO-FUNCTIONAL STATE OF THE LIVER IN TOXIC HEPATITIS

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ABSTRACT

in this study, the activity of cytochrome oxidase in toxic lesions of the liver parenchyma is shown. It was revealed that with liver damage at different periods of toxic hepatitis they lead to inhibition of cytochrome oxidase. The coefficient level corresponding to a certain amount of damaged (or intact) liver parenchyma has been established.

Key words: acute liver failure, liver parenchyma, toxic damage, ischemia, animals, mitochondria.

RELEVANCE OF THE TOPIC

At present, there is a steady increase in the number of patients with liver diseases all over the world. At the same time, various hepatic pathologies in most cases are accompanied by the development of serious complications up to the appearance of liver failure [1,2,3]. Despite the seeming simplicity of determining ARF, in practice it is not easy to diagnose this syndrome due to the often complex clinical picture, the absence of an obvious aggravating factor, difficulties in differential diagnosis with end-stage liver disease and determining the need and feasibility of specific therapeutic interventions. The diagnosis of ARF is based on the data of anamnesis, clinical picture, biochemical changes [2,4,14].

MATERIALS AND METHODS

To create an experimental model of hepatocellular insufficiency, the experiments were carried out on 110 male Vistar rats weighing 180-220 g. 3 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 [5,6] (studies after 12 hours, 18 hours, 24 hours, and 48 hours).

3. After priming with intraperitoneal injection of 0.25 ml / 100 g of CCL4 mass [6,7,8] (studies after 12 hours, 18 hours, 24 hours, and 48 hours).

The animals were sacrificed 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 (ethylenediamine tetraacetate); 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 platinum Clarke electrode on an LP-7 polarograph [9].

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- [10] (tetramethylene paraphenylenediamine) -1 µM

and cytochrome-C- [11] 1 μM. Respiratory rate was expressed in nmol O2 / minute. mg. squirrel. The prognostic coefficient (PC) was calculated according to the formula: PC = Cytochrome C - Ascorbate Na / TMPD - Ascorbate Na

A suspension of xenogenic hepatocytes was obtained by the combined Berry-Friand method modified by A.I. Archakov [12]. The degree of morphological preservation of the obtained hepatocytes was assessed by the method of light and phase-contrast microscopy, with preliminary staining with a vital dye - 0.2% trypan blue. The digital material was processed by the method of variation statistics [13].

RESULTS AND DISCUSSION

12 hours after inoculation with DL-galactosamine (Table # 1),

Table 1.

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

Период исследования	Аскорбат зависимое потребление О2	ТМФД-оксидазная активность	ЦитохромС-оксидазная активность
Контрольная группа	10,50±0,15	20,00±1,50	27,90±3,00
ДЛ-галактозамин, через (час):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,через12 час	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*

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

Polarographic studies showed 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.

Morphological examination of liver tissue revealed that already 12 hours after inoculation with DL-galactosamine (Fig. # 1) - the girder structure of the liver is somewhat disturbed, the contours of hepatocytes are indistinct.

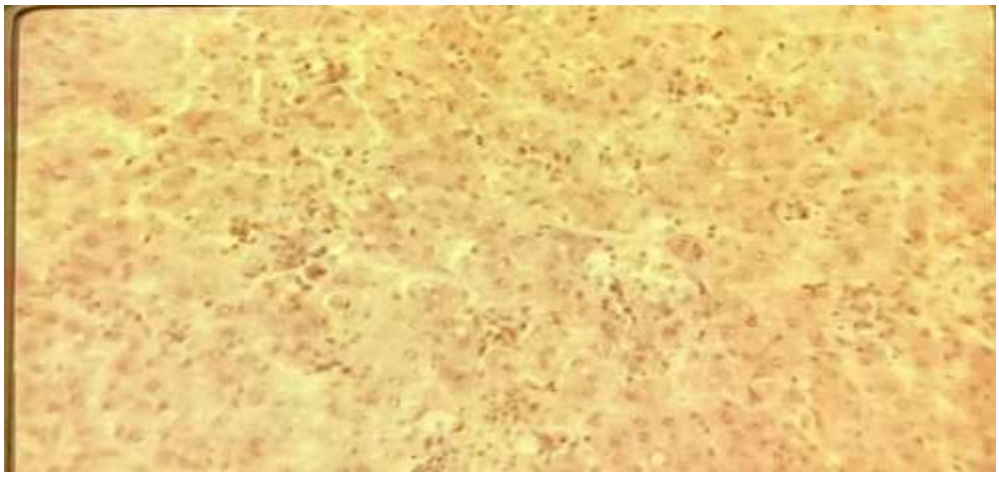

Рис.#1. Focal vacuolar degeneration of hepatocytes with perifocal lymph-leukocyte infiltration 12 hours after inoculation with DL-galactosamine. Staining with hematoxylin and eosin. Magnification x 200.

In parenchymal cells, chromatin depletion of nuclei is observed. Round-cell infiltrates with an admixture of leukocytes, scattered over the parenchyma, are ubiquitous. Infiltrates are often localized near venous vessels.

With an increase in the duration of time from the beginning of the inoculation (18 and 24 hours), these changes are aggravated, and by 48 hours (Fig. # 2) they reflect the most severe damage: total necrobiotic changes in hepatocytes.

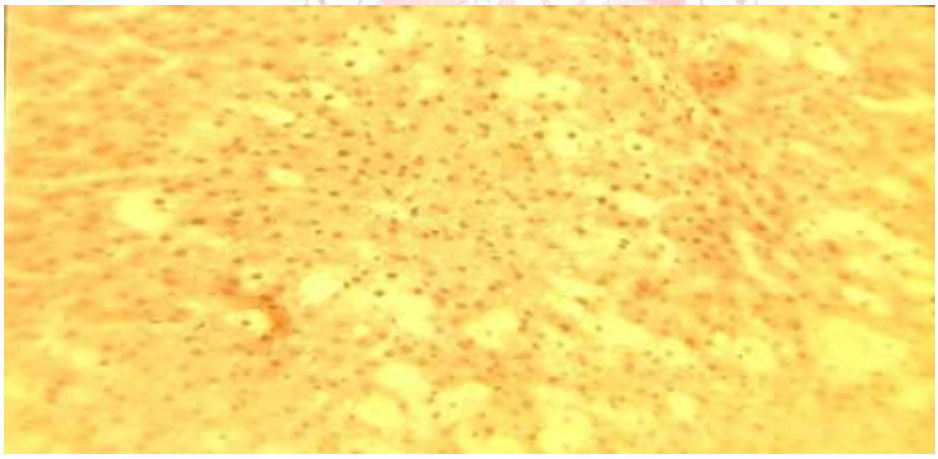

Рис.# 2. Total necrobiotic changes in hepatocytes 48 hours after inoculation with DL-galactosamine. Staining with hematoxylin and eosin. Magnification x 200.

Calculation of the number of viable liver cells shows that their number decreases with increasing time from the start of inoculation. So, after 12 hours the number of viable cells is 50% and the coefficient is 5.05, while after 48 hours it is 10-12% and the coefficient is 13.2 (Fig. # 3).

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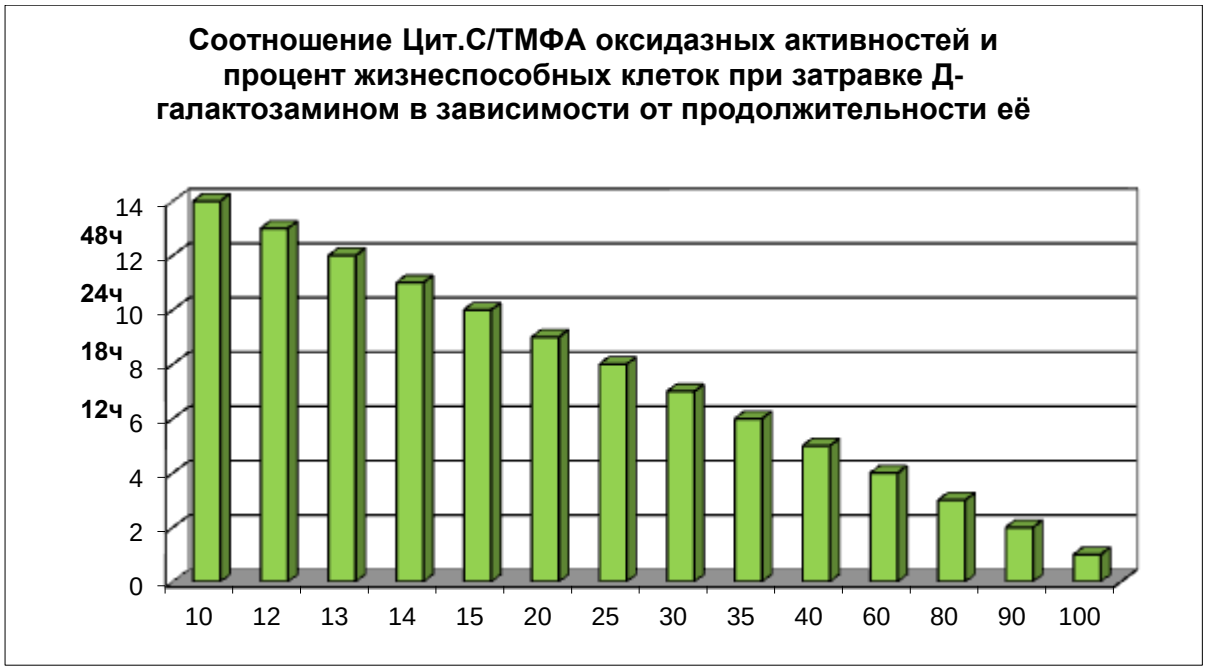
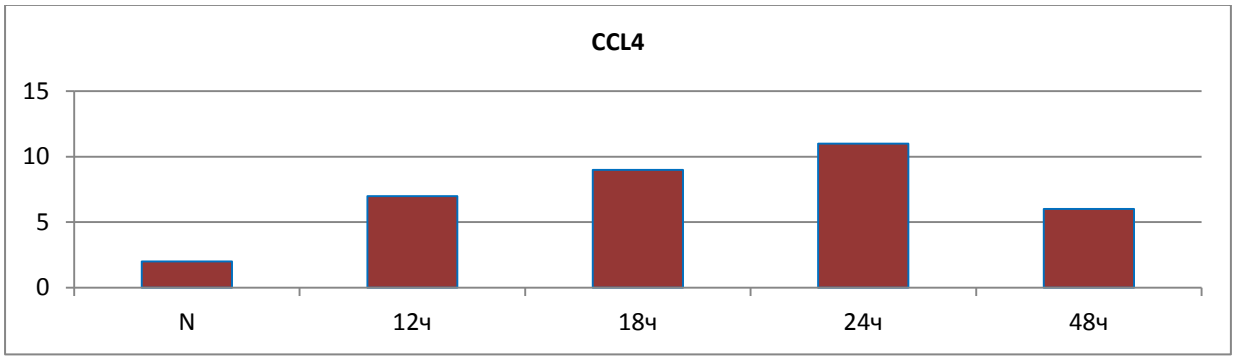


Puc.#3. Calculation of the number of viable liver cells after inoculation with DL-galactosamine (after 12, 18, 24 and 48 hours) and their ratio with the coefficient.

Observations of these animals have shown that the severity of their clinical condition increases with the length of time after priming. By 48 hours, the animals showed severe hypodynamia, bleeding, a decrease in pain sensitivity, followed by the death of 100% of the animals.

In rats that received CCL4, we revealed the same dynamics of changes in the rate of O2 consumption in various metabolic states. A distinctive feature of this model from galactosamine was a sharp activation of cytochrome C-oxidase (Table 1) (exceeding the values of intact rats by 162.4 and 136.2% after 12 and 24 hours after priming). In the group with CCL4 poisoning, it was determined (Fig. # 4) that through



Puc.#4. The value of the coefficient after seeding CCL4 (after 12, 18, 24 and 48 hours).

12 hours after priming, the value of the coefficient increases to 7.2, and after 24 hours, when the death of 50% of the animals was observed, to 10.9. In the surviving rats, after 36 and 48 hours, the value of the coefficient decreased to 7.0 and 6.0, respectively.

Morphological studies of liver tissue showed that 12 hours after priming with CCL4 (Fig. # 5), there are areas of necrosis, droplet vacuolization of hepatocytes, and fatty cells along the periphery of the lobules.

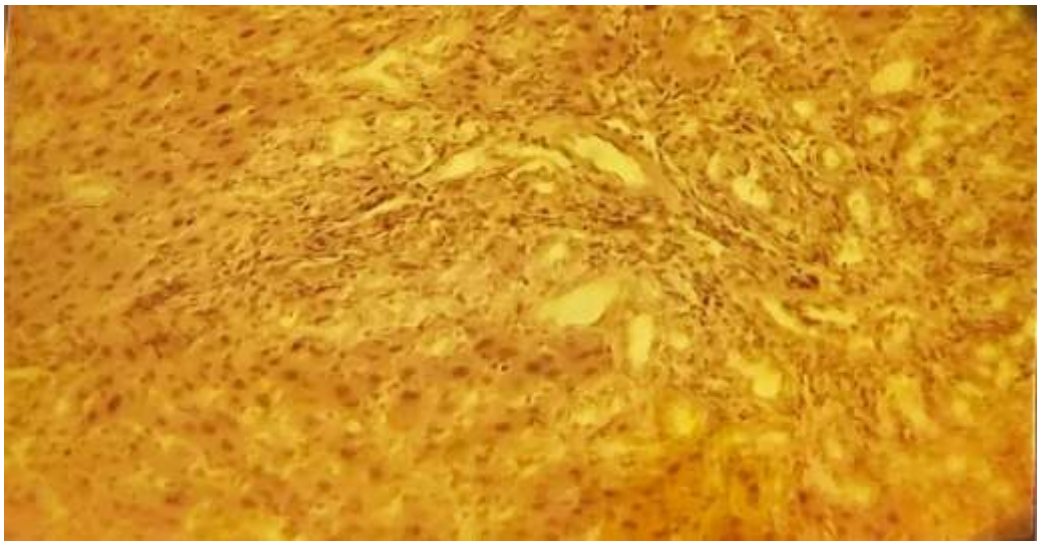


Рис.#5. There are areas of necrosis, small-drop vacuolization of hepatocytes, adiposity of cells along the periphery of the lobules 12 hours after priming with CCL4. Staining with hematoxylin and eosin. Magnification x 200.

By 24 hours (Fig. # 6), these changes are aggravated and reflect the most severe damage: total necrobiotic changes in hepatocytes

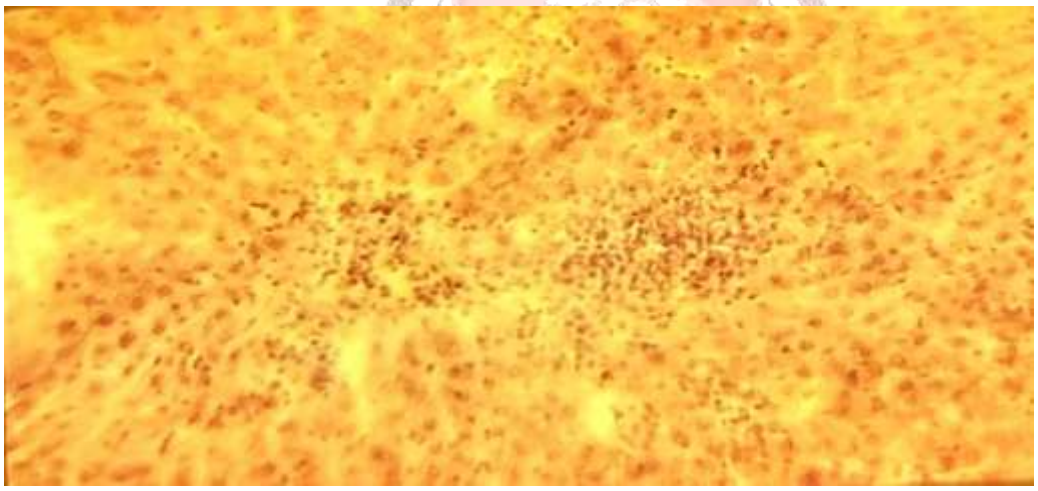


Рис.#6. total necrobiotic changes in hepatocytes 24 hours after priming with CCL4. Staining with hematoxylin and eosin. Magnification x 200.

And at 36 and 48 hours, in the preserved areas of the parenchyma, necrobiotic changes in hepatocytes decrease slightly, which corresponds to a decrease in the coefficient and there is some improvement in the condition of the animals (activity, appetite, neatness ..)

Calculation of the number of viable cells showed (Fig. # 7) that 12 hours after inoculation, 30% of intact cells remain, / coefficient 7.2 /, and after 24 hours --- 15% / coefficient -10.9 /.

Рис.#7. Calculation of the number of viable liver cells after inoculation with CCL4 (after 12 and 24 hours) and their ratio with the coefficient.

The clinical condition of rats gradually deteriorates and reaches its peak by 24 hours, when the death of 50% of the animals is noted. By the 48th hour, survivors partially restore mobility, reflexes are activated, appetite appears, bleeding decreases, and the number of viable cells also slightly increases.

Thus, the results of this series of experiments showed that the method used by us makes it possible to quantitatively assess the degree of damage to the hepatic parenchyma in case of DL-galactosamine and CCL4 poisoning. Moreover, the largest value of the coefficient corresponds to the most pronounced morphological changes, the number of viable cells and the severe clinical condition of animals.

CONCLUSIONS

1. The use of this test can serve as a method for diagnosing liver damage.

2.Allows to quantify the safety of the parenchyma.

3. Creates the prerequisites for the choice of diagnostic, therapeutic tactics in terms of the nature and extent of surgery and therapeutic methods of correction.

4. To determine the further course and prognosis of this disease in patients.

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