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ASSESSMENT OF INDICATORS OF TOXIC DAMAGE OF HEPATOCITES IN CHRONIC LIVER DISEASES

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Objective: *to study enzymes of different localization of hepatocytes in endogenous intoxication in patients with chronic liver diseases.*

Material and methods: *in patients with chronic liver diseases, the activity of hepatocyte enzymes, as well as marker enzymes of mitochondria, was determined.*

Results: *in patients with chronic diffuse liver diseases, against the background of the formation of reactive oxygen species and endogenous intoxication, hyperenzymemia was noted due to a violation of the membrane structures of hepatocyte mitochondria, as well as stimulation of macrophages in the liver, which was expressed in an increase in the concentration of cystatin C and cathepsin B in the blood. An increase in the activity of the lysosomal enzyme cathepsin B may be one of the reasons for the death of hepatocytes and the acceleration of apoptosis. Conclusions: in patients with chronic liver disease, against the background of endogenous intoxication and an increase in lipid peroxidation products, cytolytic damage to hepatocytes and hyperfermentemia occur.*

Key words: *toxic hepatitis, apoptosis, hepatocyte, liver damage, hyperenzymemia.*

Numerous studies have shown that the liver is exposed to numerous toxic agents, different in strength and duration, which leads to disorders in the macro- and micro-circulation system. With an enhanced process of catabolism or metabolic disorders, obstructive cholestatic syndrome develops. In such a situation, it is not possible to isolate any of the variants of cell death (apoptosis or necrosis) as the only one. We can only speak of a predominant character [1,2,3].

Recently, it has been established that hepatocytes express different families of receptors (TNF-R1, DR4, DR5), which are homologous intracellular regions, called the death domain (DED). The latter is activated after binding of the receptor to a specific ligand. Specific ligands for these receptors are TNF-α Fash and TRAIL. Damage to liver hepatocytes caused by activation of the TNF-R1 receptor occurs as a result of endotoxemia and ischemic, reperfusion lesions. The activating agent is tumor necrosis factor-α (TNF-α). This activates the mitochondrial pathway of cell death by activating lysosomal proteinases [5,6].

An analysis of literature data indicates that endotoxemia enhances the processes of hepatocyte apoptosis and promotes cell death. It should be noted that in the pathogenesis of the development of chronic liver disease, the leading role belongs to combined changes

in various body systems. In this regard, it is especially important to study the effect of endotoxemia on the activity of enzymes of different localization of hepatocytes in chronic liver diseases.

Purpose of the study. The study of enzymes of different localization of hepatocytes in endogenous intoxication in patients with chronic liver disease.

Material and methods. 29 patients with chronic liver disease with a predominance of cytolytic syndrome were examined. Inclusion criteria were chronic hepatitis in the reactivation phase, age over 16 years. The exclusion criteria were cardiovascular diseases, diabetes mellitus, bronchial asthma, renal pathology, oncopathology, and pregnancy. The age of the patients varied from 20 to 63 years. All patients underwent a comprehensive examination. As marker enzymes of cytoplasmic localization in blood serum, the activity of g-glutamyltransferase, alkaline phosphatase (Human kit) was determined; The activity of aconitate hydratase, TNF-α, cystatin C and α-glutathione-S-transferase was determined by the test system of BioKhimMak (Russia), the level of medium-weight molecules was studied by the method of N.I. Gabrielian (1984). The activity of the antioxidant system was assessed by the level of malondialdehyde (Stalnaya I.D., 1977), the activity of the lysosomal enzyme cathepsin B by A.A. Pokrovsky. The studies used enzyme immunoassay and biochemical analyzers of the company "Human". Statistical data processing was carried out by the method of variation statistics using Student's t-test. The results were processed with the Statistica software package.

Results and discussion. Cytokine-mediated liver damage is accompanied by the release of fragments of hepatocyte membranes, enzymes and various polypeptides into the bloodstream. Depending on the size of polypeptides and oligopeptides, molecules of average mass are captured on a spectrophotometer at different wavelengths. Otherwise they are called products of endogenous intoxication. In the patients with chronic liver disease examined by us, there is a significant increase in the amount of medium molecular weight peptides E254 by 1.7 and E280 by 1.4 times, which indicates endogenous intoxication and an increase in the level of peptides of various masses in the blood plasma.

Endotoxemia and cytokine attack on liver hepatocytes during intrahepatic cholestasis causes an "oxygen explosion" of the cell, which is also associated with an increase in the pool of medium-weight molecules against the background of dysfunction of the LPO and AOS systems, accompanied by depletion of the antioxidant system potential and a significant increase in the content of malondialdehyde in 1, 7 times ($p<0.05$). Changes in the studied parameters indicate a high sensitivity to the damaging effect of both cytokines and endotoxins of the liver, which in turn provide their clearance.

The pathological process observed in hepatocytes is accompanied by cholestasis, which in general is a violation of the synthesis, secretion and outflow of bile at the level of the bile ducts against the background of endotoxemia and an increase in the process of apoptosis of hepatocytes, resulting in the death of cells of the bile capillaries and the release of enzymes into blood plasma. In this situation, we observe a significant increase in

the activity of g-glutamyl transferase by an average of 4 times ($p<0.05$). A similar dynamics is observed with respect to the enzyme alkaline phosphatase, the activity of which in the blood plasma of the examined was 3 times higher than that of the healthy ones. The reason for this is the close location in the membrane of the epithelium of the bile ducts of the studied enzymes, which is why, when the membranes are destroyed, their activity in the bloodstream increases simultaneously and almost equally.

It is known that oxidative stress and increased formation of reactive oxygen species play an important role in cell death, including hepatocytes [4, 7]. In this situation, the main generator of reactive oxygen species are mitochondria, in which reactive oxygen species account for up to 1-2% of the total amount of molecular oxygen. Reactive oxygen species are one of the reasons for the increased permeability of mitochondrial membranes. Violation of the structural components of mitochondrial membranes against the background of endotoxemia and reactive oxygen species leads to the release of mitochondrial enzymes in the blood serum, which indicates disorders in the mitochondrial level [2,4].

Table 1

Biochemical parameters of blood in patients with CKD

Index	Healthy individuals (control), n=14	Patients with CDLD, n=29
Medium molecular weight peptides E254, arb. units	0.21±0.01	0.35±0.01*
Medium molecular weight peptides E280, arb. units	0.30±0.01	0.41±0.01*
Glutamate dehydrogenase, mmol/h/l)	15.4±0.91	35.1±3.21*
Malondialdehyde, nmol/ml	1.03±0.11	1.74±0.21*
TNF-a, pg/ml	198.4±12.6	401.6±14.1
Antioxidant activity, nmol/ml	58.4±2.13	32.4±2.61*
a-glutathione-8-transferase, ng/l	412.1±11.8	1956.0±19.2*
g-glutamyltransferase, IU/l	36.3±1.12	91.9±3.41*
Alkaline phosphatase, IU/l	68.4±5.11	226.4±11.7*

Note. * $p<0.05$ compared to control.

As can be seen from Table 1, in patients with chronic diffuse liver disease (CDLD), compared with healthy individuals, the activity of glutamate dehydrogenase increases by 2.3 times ($p<0.05$).

An imbalance between excessive production of reactive oxygen species and insufficient functioning of the antioxidant system leads to intensification of free radical oxidation. Free radicals are also formed during stimulation of Kupffer cells and sequestration of polymorphonuclear neutrophils. The protein molecule of aconitate hydratase is easily destroyed by the active form of oxygen due to the destruction of the iron-sulfur cluster in the composition of this enzyme, which leads to its inactivation [2, 3].

This allows us to consider the dynamics of aconitate hydratase as a criterion for the action of free radicals.

An analysis of the results obtained indicates a significant decrease in the activity of aconitate hydratase in the blood serum of the examined individuals by an average of 2.4 times (Table 2).

table 2

Biochemical parameters of blood in patients with CKD

Index	Healthy individuals (control), n=14	Patients with CDLD, n=29
Aconite hydratase, U/ml	0.43±0.05	0.18±0.01*
Cystatin C, ng/ml	1037.1±14.2	1754.6±12.1
Protein C, mg/l	1.14±0.18	0.85±0.07*
Fibrinogen, g/l	3.14±0.41	3.01±0.43
Cathepsin B, μmol/min/g protein	28.7±2.04	43.8±3.19*

Note. * p<0.05 compared to control.

As noted above, when Kupffer cells are stimulated, free radicals are formed, which also leads to the secretion of a low molecular weight protein, cystatin, which, being an endogenous inhibitor of cysteine proteases, regulates the activity of extracellular pool cathepsins.

The increase in the concentration of cystatin C in the blood serum of patients with chronic hepatitis noted by us is aimed at inhibiting the activity of cathepsin B or its binding. The increase in the concentration of cystatin C in the blood serum of the examined patients, which we found, indicates the possibility of using this indicator as one of the markers of macrophage stimulation in chronic liver damage.

Studies conducted in patients with chronic diffuse liver disease (CDLD) revealed a decrease in the activity of the protein C system in the blood plasma, which is apparently due to a violation of the protein-synthesizing function of the liver. In addition, a positive correlation was found between the content of protein C and fibrinogen in the blood plasma (Tables 1 and 2).

Thus, in patients with chronic diffuse liver disease against the background of the formation of reactive oxygen species and endogenous intoxication, hyperfermentemia is noted due to a violation of the membrane structures of hepatocyte mitochondria. There is also stimulation of liver macrophages, which is expressed in an increase in the concentration of cystatin C and cathepsin B in the blood. Against the background of a violation of the protein-synthesizing function of the liver, the concentration of protein C in the blood decreases. An increase in the activity of the lysosomal enzyme cathepsin B may be one of the reasons for the death of hepatocytes and the acceleration of apoptosis.

CONCLUSIONS

1. In patients with chronic liver disease, against the background of endogenous intoxication and an increase in lipid peroxidation products, cytolytic damage to hepatocytes and a state of hyperenzymemia are noted.

2. Cystatin C and cathepsin B are one of the markers of activation of the liver macrophage system.

3. The state of endotoxemia in patients with chronic liver disease is accompanied by impaired protein C synthesis.

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