Mechanisms of disorders of ischemic liver damage and ways of their correction using a new amino acid mixture based on sodium succinate and mannitol

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ABSTRACT

During the reproduction of experimental toxic hepatitis by administering heliotrine, it was found that the HIF-1 content averaged 0.101667±0.0022 ng/l. In blood plasma, the average HIF-1 values were 0.2136±0.0066 ng/l. After treatment in group I, HIF-1α indices were 0.317±0.022 ($p<0.01$), in group II – 0.404±0.031 ($p\leq0.02$), in group III – 0.365±0.026 ($p\leq0.001$), in group IV group – 0.421±0.028 ($p\leq0.001$). During the reproduction of experimental toxic hepatitis by administering heliotrine, it was found that the ALT content was on average 25.93 ± 2.91 U/l, and the AST content was at the level of 22.23 ± 1.95 U/l. The de Rits number was 1.17±0.16. Direct bilirubin was at the level of 3.90±0.44 mmol/l, indirect bilirubin – 8.10±0.8 mmol/l. Total bilirubin was 12.01±1.16 mmol/l. Moreover, the OR (odds ratio) was 0.93219976. The 95% CI (confidence interval) was 0.88765239. χ 2= 0.9633286 (Wilkonson test). The Mann-Whinney test (U test) was 0.87219981 at $p<0.05$.

Conclusions: The developed amino acid mixture is superior to traditional methods of treatment (Infezol) in terms of the effectiveness of influence on the development and course of experimental toxic hepatitis, which is proved by the study.

Keywords: toxic hepatitis; amino acid mixtures; heliotrin intoxication; ischemia; hypoxia; Infezol

Relevance. Despite the achievements of modern hepatology, non-infectious and infectious liver diseases remain common causes of disability and mortality among the population. Every year, about 25 thousand people suffering from toxic hepatitis are observed in the hepatological centers of Uzbekistan. In 2020, the number of patients increased by 1.6% compared to 2019. At the same time, toxic damage to the liver by various chemicals (alcohol, carbon tetrachloride, drugs) contributes to the occurrence and progression of somatic diseases, which significantly affects the health of patients [8-10, 15].

Acute and chronic intoxication with hepatotoxins leads to a significant change in the cyto- and histoarchitectonics of the liver, disruption of normal metabolism in tissues. The development of toxic hepatitis is accompanied by dystrophy and necrosis of hepatocytes, massive formation of portocaval anastomoses, as a result of which both the synthetic function of the liver and its ability to neutralize foreign substances are impaired [4, 12, 14].

One of the leading syndromes that increase the severity of the course of toxic liver damage is the syndrome of endogenous intoxication. This is due to the breakdown of hepatic parenchyma cells and the accumulation of toxic products in the periocellular space, followed by their entry into the bloodstream, which leads to disruption of cellular metabolism and weakening of the regulatory and adaptive functions of both the liver itself and the whole organism as a whole. Oxidative stress occurring against the background of toxic hepatitis is considered as a complex response of the body to aggression from the environment, which is accompanied by pronounced nonspecific changes in biochemical parameters [1-3, 5, 17].

The best means of influencing metabolic homeostasis are mixtures of pure amino acids made according to certain recipes, since protein synthesis occurs only from free amino acids. Nitrogenous preparations used for parenteral nutrition contain all essential amino acids in sufficient quantities, the so-called replaceable nitrogen (glycine, etc.) [6, 11, 13]

The advantages of amino acid mixtures over protein hydrolysates are obvious, because they are easily controlled by their amino acid composition, do not contain humic substances, ammonia and other undesirable components. Many years of experience in the use of amino acid preparations as a basic method of intensive therapy aimed at eliminating gross violations of water-electrolyte and protein metabolism, prevention and treatment of multiple organ failure, has shown its high effectiveness in the complex treatment of severe diseases of various etiologies [7, 16, 18-20]

Currently, there are a number of drugs widely used in medicine that are balanced in terms of the content of essential and interchangeable amino acids – Infezol 40, Infezol 100 (Berlin-Chemi, Germany), Aminoplasmal E – 5%, 10% (B. Brown, Germany), Aminosol – 600, 800, KE ("Chemopharm", Yugoslavia).

The Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan has developed a blood substitute containing amino acids and an antioxidant complex with a wide spectrum of action, capable of protein synthesis, mobilization of energy and plastic resources, optimization of the activity of physiological systems, acceleration of recovery processes in severe diseases of various etiologies associated with disorders of protein and energy metabolism. The purpose of the study was to conduct an open randomized study to examine the effectiveness of a new amino acid mixture based on sodium succinate and mannitol for the course of experimental toxic hepatitis. Scientific novelty for the first time, a study of a new amino acid mixture for the course of toxic hepatitis will be conducted on the basis of biochemical research methods.

Materials and methods of research. To achieve this goal, a model of toxic hepatitis was reproduced using the example of heliotrine intoxication. Acute heliotrine intoxication was reproduced by a single injection of a subcutaneously sublethal dose of heliotrine prepared at the rate of 40 mg per 100 g of body weight to rats. Toxic hepatitis is reproduced by subcutaneous administration of heliothrin (25 mg/ 100 g). The material for the study is venous blood. The indicators of protein balance were studied: total serum protein, albumin and globulin and biological materials (ALT, AST, bilirubin and alpha-amylase by biochemical analysis using HUMAN test systems (Germany) on semi-automatic biochemical analysis BA88A (Mindray, China). Protein fractions will be determined by the turbidimetric method according to the generally accepted method. The content of HIF-1 in the blood was determined by the enzyme immunoassay. The animals were divided into equal groups:

Group I – before reproduction of heliotrine intoxication (intact)

group II (control) – with heliotrine intoxication,

Group III (control, comparison) – with heliotrine intoxication after administration of the comparison drug "Infezol 40", within 5 days 24 hours after the last administration; Group IV (main, experimental) – animals with heliotrine intoxication after the introduction of a new amino acid blood substitute, within 5 days 24 hours after the last administration. Statistical processing was performed using the Student-Fisher criterion, the nonparametric Mann-

Winnie criterion, and the Kraskes-Wallis criterion.

The results of the study. During the reproduction of experimental toxic hepatitis by administration of heliotrin, it was found that the HIF-1 content was on average equal to 0.101667±0.0022 ng/l. In blood plasma, the average HIF-1 values were 0.2136±0.0066 ng/l. Such indicators are explained by the effect of heliotrin on the liver and, above all, on hepatocytes, in which mitochondria are deficient in oxygen. Thus, HIF-1 acts as an early biomarker of tissue oxygen deficiency, and since it causes angiogenesis, the amplification of this gene in experimental animals with ischemia may promote vascular proliferation necessary for oxygenation. On the contrary, since HIF-1 promotes the survival and proliferation of cancer cells due to its angiogenic properties, inhibition can potentially prevent the spread of cancer. With the growing understanding of the HIF-1 pathway, inhibition and stimulation of its transcriptional activity by small molecules is now an attractive target.

As is known, the HIF-1a subunit also contains two transactivation domains (TAD) that regulate HIF-1 target genes. CREB-binding protein (CBP) and p300, two HIF-1 transcription coactivators, interact with the carboxyl-terminal transactivation domain (C-TAD) of HIF-1α. Both activators are necessary for HIF-1 transcription and, therefore, are targets for the regulation of HIF-1 expression; inhibition of HIF-1-C-TAD interactions by proline hydroxylation suppresses HIF-1 gene expression, preventing normal transcription and translation. HIF-1ß contains only one such analogous region, which is not needed for the complex function HIF-1. Recent reports show that HIF-1ß is identical to a previously discovered vertebrate protein, the nuclear translocator of the aryl hydrocarbon receptor (ARNT). HIF-1 is the main regulator of oxygen homeostasis in cells. As a transcription factor, it influences and regulates the expression of dozens of genes involved in maintaining homeostasis when oxygen concentration changes. One of the important functions of HIF-1 is to promote angiogenesis; HIF-1 directs the migration of mature endothelial cells into a hypoxic environment. This is done by HIF-1 regulation of vascular endothelial growth factor (VEGF) transcription. VEGF is the main regulator of angiogenesis, which promotes the migration of endothelial cells towards the hypoxic region. During hypoxia, HIF-1 binds the regulatory region of the VEGF gene, inducing its transcription and initiating its expression. Such endothelial cells eventually help to form new blood vessels, supplying this area with oxygenated blood. During the reproduction of experimental toxic hepatitis by administration of heliotrin, it was found that the content of c and, OR (odds ratio) was 0.93219976. CI (confidence interval) 95% was 0.88765239. $\chi 2$= 0.9633286 (Wilconson criterion). The Mann-Winnie criterion (criterion U) was 0.87219981 at $p<0.05$. These indicators indicate that the indicators of protein balance are directly dependent on oxygen deficiency caused by heliotrin. However, the ALT level is an unreliable marker of the pathological process in the liver. This is primarily due to the peculiarity of the laboratory method, when not the actual level of the enzyme is determined, but its catalytic activity, the rate of the catalytic reaction. Thus, the amount of the enzyme is determined indirectly. The results obtained indicate that as a result of treatment, the indicators of total bilirubin in group IV significantly improved. The dynamics of ALT was positive in group IV, who received the developed amino acid mixture, there was no significantly positive dynamics of ALT and AST indicators in group III, who received Infezol. In general, we can say that in the case of toxic hepatitis with a 2-fold or more increase in ALT activity, intravenous Infezol therapy with a simple cancellation of the damaging factor is not effective enough. In addition, the restoration of liver detoxification function by the end of the course of treatment, which was observed in the study group receiving the developed amino acid mixture, can be interpreted as the most important indicator of the effectiveness of therapy, speaking in favor of metabolic therapy. Of interest is the use of the recommended amino acid mixture, which was unambiguously positive in all values — a decrease in cytolysis

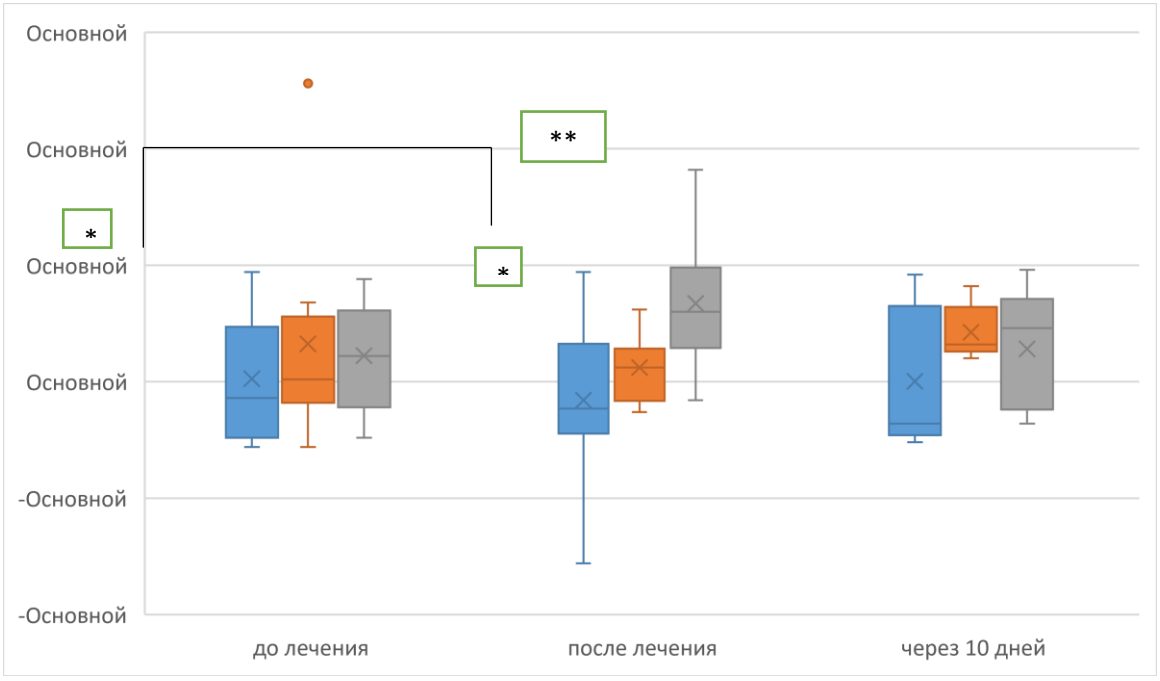
and cholestasis and an increase in detoxification function of the liver.

Table 1. Statistical data on the main parameters of toxic liver damage

Groups	1 Group	2 Group	3 Group	4 Group
95% CI	0,45-4,35	1,36-6,35	0,63-6,21	0,32-6,27
OR	0,86549908	0,75423009	0,87661024	0,93219976
χ 2 (Wilconson criterion)	0,7988014	0,7210023	0,8321098	0,9633286
U (Mann-Winnie criterion)	0,81230091	0,65001459	0,82109273	0,87219981
The Kraskes-Wallis criterion	0,75800213	0,83400219	0,87201108	0,91005467
P (Student-Fisher criterion)	<0,05	=0,03	<0,01	≤0,001

CI – confidence interval
OR – odds ratio

Fig. 2. Values of the Kraskes-Wallis criterion in toxic liver damage by the example of heliotrine intoxication.



*-p≤0,05
**-p≤0,01

According to the Kraskes-Wallis criterion, the largest precentile is Q1=132, and the smallest is Q4=78, which corresponds to p≤0.05. The application of the Mann-Whitney and Wilcoxon criteria was justified, since we estimated the difference between the medians of the two general aggregates. The advantage of the Kraskes-Wallis criterion is that it can be applied even when only rank indicators are available to the researcher.

Conclusions: Summarizing the above, hypoxia-inducible factor 1 (HIF-1a) is an important pathogenetic link in the development of oxygen

deficiency and its deficiency at an early stage can serve as an important diagnostic biomarker of toxic hepatitis, including those caused by heliotrin. Since the chemical composition belongs to pyrolyzidine alkaloids, and as is known, its precursor is cadeverdine, which is oxidized to gamma-aminobutyric aldehyde to form non-amino alcohols with monobasic non-amino acids. The developed amino acid mixture is effective the effects on the development and course of experimental toxic hepatitis are superior to traditional methods of treatment (Infezol), which is proved by the study.

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