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

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Abstract. The aim of the study. Conduct an open-label randomized study to study the effectiveness of a new amino acid mixture based on sodium succinate and mannitol in the course of experimental toxic hepatitis. **Materials and research methods.** Acute heliotrin intoxication was reproduced by a single subcutaneous administration of a sublethal dose of heliotrin to rats, prepared at the rate of 40 mg per 100 g of body weight. Toxic hepatitis was reproduced by subcutaneous administration of heliotrin (25 mg / 100 g). The material for the study is venous blood. Indicators of protein balance were studied: total serum protein, albumin and globulin and biological materials (ALT, AST, bilirubin and alpha-amylase by biochemical analysis with HIF-1 content in the blood was determined by enzyme immunoassay. Animals were divided into equal groups: Group I - before reproduction of heliotrin intoxication (intact), group II (control) - with heliotrin intoxication, group III (control, comparison) - with heliotrin intoxication after the introduction of the reference drug "Infezol 40", within 5 days 24 hours after the last injection; IV group (main, experimental) - animals with heliotrin intoxication after the introduction of a new amino acid blood substitute, within 5 days 24 hours after the last injection Statistical processing was performed using the Student-Fisher test, the nonparametric Mann-Winney test, the Kraskes-Wallis test. **Research studies**. During the reproduction of the experimental toxic hepatitis by the introduction of heliotrin, it was found that the content of HIF-1 was on average 0.101667 ± 0.0022 ng / l. In blood plasma, the mean HIF-1 values were 0.2136 ± 0.0066 ng / L. After treatment, in group I, HIF-1α values were 0.317 ± 0.022 ($p < 0.01$), in group II - 0.404 ± 0.031 ($p \leq 0.02$), in group III - 0.365 ± 0.026 ($p \leq 0.001$), in group IV group - 0.421 ± 0.028 ($p \leq 0.001$). During the reproduction of experimental toxic hepatitis by the introduction of heliotrin, 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 were at 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. The total bilirubin was 12.01 ± 1.16 mmol / L. Moreover, OR (odds ratio) was 0.93219976. The 95% CI (confidence interval) was 0.88765239. $\chi 2 = 0.9633286$ (Wilconson test). Mann-Winney 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

Introduction. Despite the advances in modern hepatology, non-infectious and infectious liver diseases remain common causes of disability and mortality among the population. About 25 thousand people suffering from toxic hepatitis are observed annually 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 onset and progression of somatic diseases, which significantly affects the health of patients [1-3].

Acute and chronic intoxication with hepatotoxins leads to a significant change in the cyto- and histoarchitectonics of the liver, disruption of normal tissue metabolism. 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].

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 disintegration of the cells of the hepatic parenchyma and the accumulation of toxic products in the pericellular 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. The oxidative stress arising 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 [5-9].

The best means of affecting metabolic homeostasis are mixtures of pure amino acids, compiled according to certain formulations, since protein synthesis occurs only from free amino acids. Nitrogen preparations used for parenteral nutrition contain all essential amino acids in sufficient quantities, the so-called nonessential nitrogen (glycine, etc.) [10-15]

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 efficiency in the complex treatment of severe diseases of various etiologies [16-20]

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

At the Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan, a blood substitute has been developed containing amino acids and an antioxidant complex, with a wide spectrum of action, capable of synthesizing proteins, mobilizing energy and plastic resources, optimizing the activity of physiological systems, accelerating recovery processes in severe diseases of various etiologies associated with disorders of protein-energy metabolism.

The aim of the study. Conduct an open-label randomized study to study the effectiveness of a new amino acid mixture based on sodium succinate and mannitol in the course of experimental toxic hepatitis.

Materials and research methods. To achieve this goal, a model of toxic hepatitis was reproduced using the example of heliotrin intoxication.

Acute heliotrin intoxication was reproduced by a single subcutaneous administration of a sublethal dose of heliotrin to rats, prepared at the rate of 40 mg per 100 g of body weight. Toxic hepatitis was reproduced by subcutaneous administration of heliotrin (25 mg / 100 g). The material for the study is venous blood. The indicators of protein balance were investigated: total blood 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). fraction 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 heliotrin intoxication (intact)

Group II (control) - with heliotrin intoxication,

Group III (control, comparison) - with heliotrinic intoxication after administration of the reference drug "Infezol 40", within 5 days, 24 hours after the last injection;

Group IV (main, experimental) - animals with heliotrin intoxication after the introduction of a new amino acid blood substitute, within 5 days, 24 hours after the last injection. Statistical processing was carried out using the Student-Fisher test, the nonparametric Mann-Winney test, the Kraskes-Wallis test.

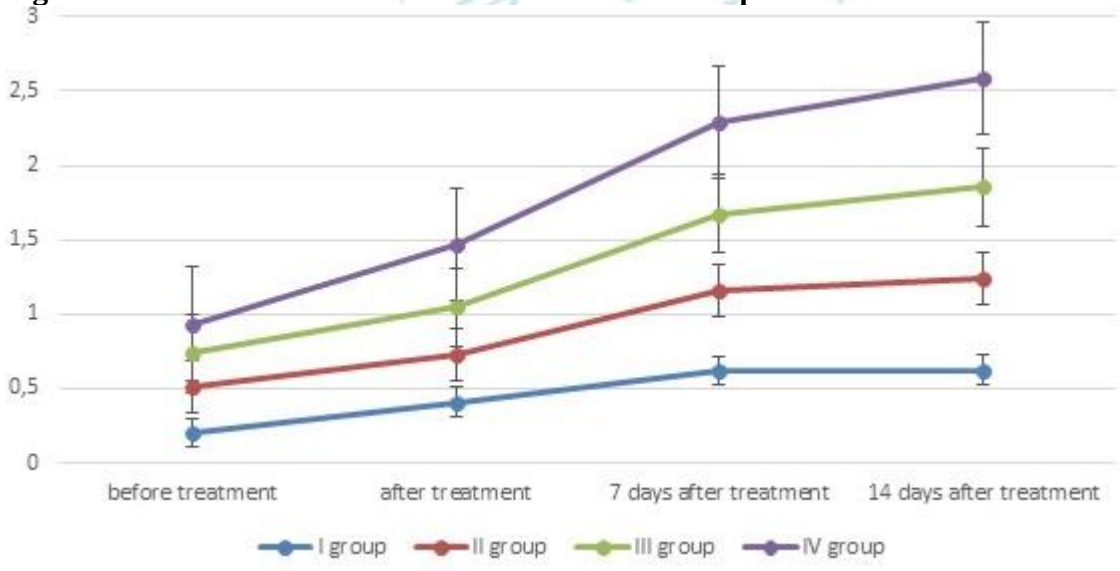
Research results. During the reproduction of experimental toxic hepatitis by the introduction of heliotrin, it was found that the HIF-1 content was on average 0.101667 ± 0.0022 ng / L. In blood plasma, the mean HIF-1 values were 0.2136 ± 0.0066 ng / L. Such indicators are explained by the effect of heliotrin on the liver and, first of all, on hepatocytes, in which mitochondria are deficient in oxygen. For example, HIF-1 acts as an early biomarker of tissue oxygen deficiency and, since it induces angiogenesis, an upregulation of this gene in experimental animals with ischemia may promote vascular proliferation required for oxygenation. In contrast, since HIF-1 promotes the survival and proliferation of cancer cells due to its angiogenic properties, inhibition could 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 goal. As is known, the HIF-1α subunit also contains two transactivation domains (TAD) that regulate the target genes of HIF-1. CREB-binding protein (CBP) and p300, two coactivators of HIF-1 transcription, interact with the carboxy-terminal transactivation domain (C-TAD) of HIF-1α.

Both activators are required for HIF-1 transcription and, therefore, are targets for the regulation of HIF-1 expression; inhibition of HIF-1α C-TAD interactions by hydroxylation of proline suppresses HIF-1 gene expression, preventing normal transcription and translation. HIF-1β contains only one such analogous region, which is not required for the complex function of HIF-1. Recent reports indicate that HIF-1β is identical to a previously discovered vertebrate protein, the aryl hydrocarbon receptor nuclear translocator (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 accomplished through 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 area. During hypoxia, HIF-1 binds the regulatory region of the VEGF gene, inducing its transcription and initiating its expression. These endothelial cells ultimately help form new blood vessels by supplying oxygenated blood to the area.

Fig. 1. The content of HIF-1α in the blood serum of experimental animals.



During the reproduction of experimental toxic hepatitis by the introduction of heliotrin, 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 were at 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. The total bilirubin was 12.01 ± 1.16 mmol / L. Moreover, OR (odds ratio) was 0.93219976. The 95% CI (confidence interval) was 0.88765239. χ 2 = 0.9633286 (Wilconson test). Mann-Winney test (U test) was 0.87219981 at $p < 0.05$. These

indicators indicate that the indicators of protein balance are in direct proportion to 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 enzyme is determined indirectly.

The results obtained indicate that as a result of treatment, the indicators of total bilirubin significantly improved in group IV. The dynamics of ALT was positive in group IV, who received the developed amino acid mixture, there was no reliably positive dynamics of ALT and AST 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 therapy with Infezol with a simple cancellation of the damaging factor is not effective enough. In addition, the restoration of the detoxification function of the liver 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, which speaks in favor of metabolic therapy .. Of interest is the use of the recommended amino acid mixture, which was unequivocally positive for all values - a decrease in the indicators of cytolysis and cholestasis and an increase in the detoxification function of the liver.

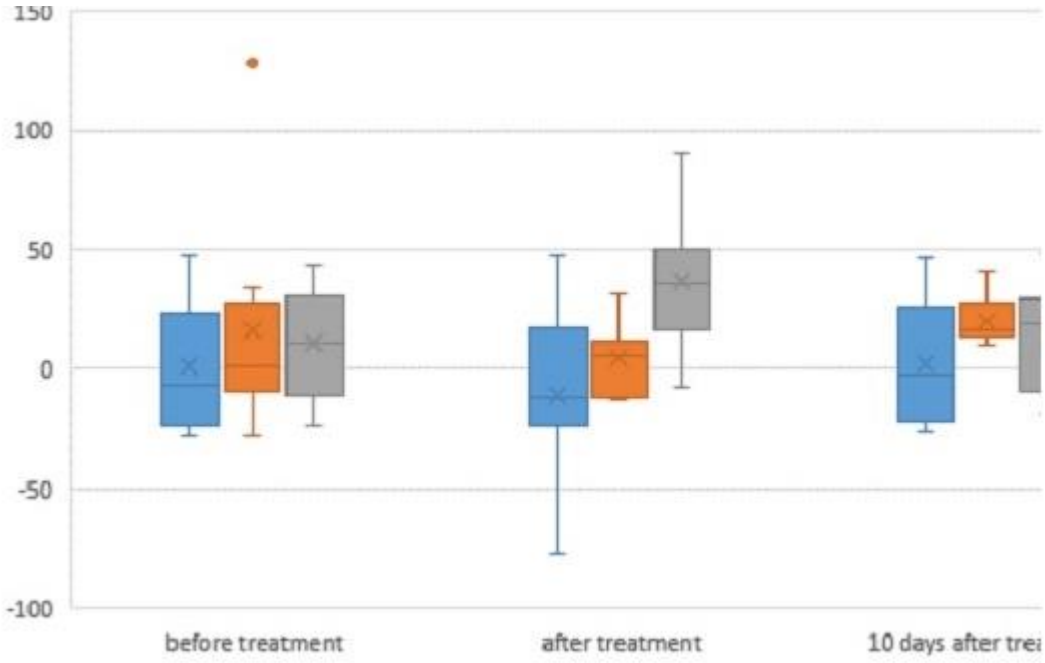
Table 1. Statistical data on the main parameters of toxic liver damage

Groups	I group	II group	III group	IV 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 test)	0,7988014	0,7210023	0,8321098	0,9633286
U (Mann-Winney test)	0,81230091	0,65001459	0,82109273	0,87219981
The Kraskes-Wallis criterion	0,75800213	0,83400219	0,87201108	0,91005467
p (Student-Fischer test)	<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 heliotrin intoxication.



*****-p≤0,05
**-p≤0,01

Conclusions: Summarizing the above, hypoxia-inducible factor 1 (HIF-1α) 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 that caused by heliotrin, since by its chemical composition it belongs to pyrolizidine alkaloids, and as you know, its precursor is cadeverdine, which is oxidized to gamma-aminobutyraldehyde with the formation of noninic alcohols with monobasic noncynic acids. 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.

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