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INFLUENCE OF A NEW AMINO ACID BLOOD SUBSTITUTE ON THE COURSE OF INTOXICATION CAUSED BY HELIOTRIN IN EXPERIMENT

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ABSTRACT

The work aimed to study the effect of a new amino acid blood substitute during heliotrine intoxication. Experimental studies were performed on 60 rats weighing 180-220g. on a model of heliotrine intoxication in comparison with the drug "Infezol-40". We studied: biochemical parameters, indicators

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of "endogenous intoxication" (EI), lipid peroxidation (LP), the activity of enzymes of the antioxidant system (AOS), and general antioxidant status.

The study results showed that the "new amino acid infusion drug" had a good detoxification effect that reduces endogenous intoxication indicators and restores the biochemical parameters of the liver's functional and metabolic parameters during heliotrin intoxication. The use of a "new amino acid infusion drug" for heliotrin intoxication reduced the activity of hyperlipoperoxidation processes and restored the activity of AOS enzymes, antioxidant status, showing a more pronounced antioxidant effect compared to the drug "Infezol-40."

Keywords: "New amino acid infusion drug", Amino acids, Heliotrin intoxication, Lipid peroxidation, Antioxidant system, General antioxidant status.

抽象的

这项工作旨在研究一种新的氨基酸血液替代品在日葵碱中毒期间的作用。对 60 **只体重** 180-220g **的大鼠**进行了实验研究。与药物“Infezol-40”**相比的日葵碱中毒模型。我**们研究了：生化参数、“**内源性中毒**”（EI）**指**标、脂质过氧化（LP）**、抗氧化系**统（AOS）**酶的活性和一般抗氧化状**态。

研究结果表明，“**氨基酸**输液新药”**具有良好的解毒作用，可降低内源性中毒指**标，恢复日葵素中毒过程中肝脏功能和代谢参数的生化参数。使用一种“**新型氨基酸**输液药物”**治**疗 heliotrin **中毒降低了超脂**质过氧化过程的活性，并恢**复了** AOS **酶的活性，抗氧化状**态，与药物“Infezol-40”**相比**，显示出更明显的抗氧化作用。

关键词：“**氨基酸**输液新药”，**氨基酸**，**日葵素中毒**，**脂**质过氧化，抗氧化系统，一般抗氧化状态。

MATERIALS AND METHODS

The experiments were carried out on 60 rats weighing 180-220 g. on the model of heliotropin intoxication. Acute heliotrin intoxication was caused - the rats were injected once subcutaneously with a sublethal dose of heliotrin prepared at the rate of 40 mg per 100 g of body weight [5, p. 30-34].

The action's effectiveness was studied compared with the blood substitute "Infezol-40," widely used in medicine. The animals were divided into equal groups: I - intact, II - control (after heliotrin intoxication), III - with heliotrin intoxication after infusion of Infezol-40, IV - with heliotrin intoxication after the infusion of a new amino acid blood substitute. Blood substitutes were administered for five days at a dose of 5 ml/kg.

During the experiment, the following indicators were studied: blood biochemical parameters - the concentration of ALT, AST, total and direct bilirubin, urea and creatinine were determined in blood serum - on a semiautomatic biochemical analyzer Mindray BA88 (China), using the test systems "Human" (Germany) [6, p. 1-750];

endogenous intoxication (EI) - the level of medium molecules (SM) in plasma and erythrocytes, the concentration of oligopeptides in plasma and erythrocytes, the sorption capacity of erythrocytes (SEE) [7, p. 11-14; 8, c. 25-29];
lipid peroxidation (LPO) - malondialdehyde (MDA), diene conjugates (Dcon) and diene ketones (Dket). The state of the antioxidant system (AOS) - the activity of AOS enzymes: superoxide dismutase (SOD), catalase (QD) [9, p. 42-45; 10, c. 107-113], glutathione peroxidase (GPO), glutathione reductase (GR) [11, p. 144-148]. Measurements in the study of endogenous intoxication indicators, the intensity of LPO processes, and the activity of AOC enzymes were performed on a UNICO2800 spectrophotometer (United Products and Instrument, Inc., USA).
The total antioxidant status was determined in blood plasma by the enzyme immunoassay (ELISA) and the use of Cayman test systems (USA). Measurements were performed at 405 nm on an MR96 microplate photometer (Mindray, China); Statistical processing of the data obtained was carried out using the Student's t-test and Mann-Whitney the Excel and Biostat programs. The criterion for statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

The experiments showed that after heliotrin intoxication, the animals' general condition was severe; the animals were inactive and lethargic.
The study of biochemical parameters, as can be seen from Table 1, showed that the level of ALT and AST increased 4.5 times (p1 <0.0001) and 5.6 times (p1 <0.0001) and, accordingly, the content of total bilirubin increased by 3.0 (p1 <0.0001), direct by 3.7 times (p1 <0.0001), which indicated the development of cytolysis and cholestasis. The urea content increased slightly by 1.1 (p1 = 0.589) times, and the creatinine concentration by 1.2 times (p1 <0.0001) (Table 1).
After introducing blood substitutes, against the background of a strong suppression of the liver's functional activity, the condition of the animals improved, lacrimation, salivation stopped, and the animals became active.

Table 1

CHANGES IN CERTAIN INDICATORS DURING HELIOTRIN INTOXICATION AND AFTER BLOOD SUBSTITUTE INFUSION IN RATS (M±m)

Indicators		Intact, n=15	Intoxication, n=15	1 hour after infusion:	
				«Infezol -40» , n=15	amino acid blood substitute, n=15
ALT, u / l		21,0±1,4	95,4±2,3	31,8±1,7	26,5±1,5
AST, u / l		14,6±1,2	81,2±2,0	25,1±1,5	23,9±1,4
Bilirubin,	straight line, μmol / l	3,7±0,4	13,6±0,5*	4,1±0,4^	3,6±0,3^
	total μmol / l	16,5±1,3	50,3±2,0*	20,4±1,6^	19,5±1,4^
Urea mmol / l		4,7±0,8	5,4±1,0	5,2±1,1	5,0±0,8
Creatinine mmol / l		66,5±1,7	81,5±2,1*	74,5±1,9	67,0±1,8^

*Note: * - significance of the difference (p <0.05) when comparing the results with the initial data; ^ the same (p <0.05) when comparing the results with the data obtained after heliotrin*

intoxication; # - the same (p <0.05) when comparing the results with the data obtained after infusion of "Infezol-40".

After infusion of the drug "Infezol-40" (group III), the concentration of ALT significantly decreased by 3.0 times (p2 <0.0001), and AST - by 3.2 times (p2 <0.0001), total and direct bilirubin by 2.5 and 3.3 times (p2 <0.0001), which were restored, approaching the values of the norm. Urea and creatinine levels decreased slightly.

In group IV, after the infusion of an amino acid blood substitute, biochemical parameters decreased as follows: the level of ALT, AST by 3.6 and 3.4 times (p2 <0.0001), total and direct bilirubin by 2.6 and 3.8 times (p2 <0.0001) respectively. Moreover, the concentration of ALT after the new blood substitute was 1.2 times (p3 <0.05) (16.7%) lower than in group III, in which the reference drug "Infezol-40" was used. The creatinine level was 1.1 times (p1 <0.01) lower, and the level of urea was practically the same in both groups III and IV.

In the study of lipid peroxidation (LPO) with heliotrin intoxication, an increase in the level of MDA in the plasma occurred comparable to the change in this indicator in erythrocytes: 2.7 and 2.5 times (p1 <0.0001), respectively. In the study of lipid peroxidation (LPO) with heliotrin intoxication, an increase in the level of MDA (malonic dialdehyde) in the plasma occurred comparable to the change in this indicator in erythrocytes: 2.7 and 2.5 times (p1 <0.0001), respectively. The concentration of diene ketones increased 2.7 times, and diene conjugates 2.5 times (p1 <0.0001) (Table 2).

Hyperlipoperoxidation led to an imbalance in the antioxidant defence system. The activity of enzymes of the antioxidant system during intoxication changed as followed: SOD activity in plasma significantly decreased 1.8 times (p1 <0.05), LPO 2.9 times (p1 <0.0001), catalase activity decreased 1.7 times (p1 <0.0001), LP increased 1.2 times: SOD activity in plasma significantly decreased by 1.8 times (p1 <0.05), LP by 2.9 times (p1 <0.0001), catalase activity decreased by 1.7 times (p1 <0.0001), LP increased by 1.2 times.

Table 2

CHANGE IN INDICATORS OF LIPID PEROXIDATION AND ANTIOXIDANT SYSTEM DURING HELIOTRIN INTOXICATION AND AFTER THERAPY (M ± m)

Indicators	Intact, n=15	Intoxication, n=15	1 hour after infusion:	
			«Infezol - 40», n=15	Amino Acid Blood Substitute, n=15
Antioxidant system (AOS)				
General antioxidant status, conv. units (mm)	1,65±0,09	0,90±0,07*	1,14±0,08*^	1,38±0,08^#
CT er. Nm / mg Hbx min	45,2±1,5	26,4±1,1	33,5±1,3*^	43,5±1.4
SOD er. meth.ed / mg Nb	2,2±0,4	1,2±0,1*	1,8±0,1	2,3±0,2^

LP sol., MkM NADFN2 / minxg Nb	2,6±0,1	3,1±0,2*	2,8±0,1^	2,5±0,1^
LPO sol. Meth. ed / minxmg Nb	0,35±0,04	0,12±0,01*	0,19±0,02^	0,26±0,02^
Lipid Peroxidation (LPO)				
MDA pl., Nm / ml pl	1,2±0,07	3,2±0,09*	2,0±0,08*^	1,3±0,07^#
MDA sol., Nm / ml pl	0,6±0,05	1,5±0,07*	1,0±0,05^	0,7±0,05*^#
Dket, otn. ed.	0,22±0,06	0,62±0,09*	0,34±0,04^	0,23±0,03*
Dket, otn. ed.	1,3±0,08	3,2±0,11*	1,7±0,09^	1,4±0,08^

Note: Designations were the same as in table 1.

The administration of Infezol-40 led to a significant decrease in lipid peroxidation indicators. Thus, the level of MDA in plasma and erythrocytes was significantly lower compared with the indicators for heliotrin intoxication by 1.6 and 1.5 times (p2 <0.0001), diene ketones and diene conjugates by 1.8 and 1.9 times (p2 <0.05; p2 <0.0001).

The activity of AOS enzymes after infusion of Infezol-40 was significantly higher compared with the indicators of the control group: catalase - 1.3 times (p2 <0.0001), SOD - 1.5 times (p2 <0.02) and GPO 1.6 times (p2 <0.01). The LP activity after the application of Infezol-40 decreased by 1.1 times (p2 <0.05). The use of the "Infezol-40" drug also led to an increase in the general antioxidant status by 1.3 times (p2 <0.05).

After the infusion of a new blood substitute, a decrease in the values of indicators characterizing the intensity of LPO was also observed: the level of MDA of plasma and erythrocytes was significantly lower relative to the control group by 2.5 and 2.1 times (p2 <0.0001), respectively, diene ketones by 2.7 times (p2 <0.0001) and diene conjugates by 2.3 times (p2 <0.0001). At the same time, the level of MDA in plasma and erythrocytes was 1.5 times (p3 <0.0001) and 1.4 times (p3 <0.0001), respectively, significantly lower than after using the medication. "Infezol-40". Compared with group III, after the infusion of a new blood substitute, the content of diene ketones and conjugates was 1.5 times (p3 <0.05) and 1.2 times (p3 <0.02) lower.

The infusion of the new blood substitute had a positive effect on the activity of AOS enzymes. There was an increase, in comparison with the indicators of animals with heliotrope intoxication, in the activity of catalase by 1.6 times (p2 <0.0001), SOD by 1.9 times (p2 <0.0001), LPO by 2.2 times (p2 <0.0001), and the LP activity decreased 1.2 times (p2 <0.05). Compared to the result from the introduction of the reference drug "Infezol-40", after the use of the new blood substitute, the catalase activity was 1.3 times higher (p3 <0.0001), SOD - 1.3 times (p3 <0.05), LPO - 1, 4 times (p3 = 0.02) and HR decreased by 1.1 times (p2 <0.05). After introducing a new amino acid preparation, the general antioxidant status, compared with heliotrope intoxication, increased 1.5 times, which was 1.2 times higher than in group III (by 21.1%).

The results of studying the antioxidant status also confirmed the presence of the antioxidant effect of the amino acid blood substitute, which showed

that it was 1.2 times higher (p3 <0.05) than after the use of the "Infezol-40" drug (Table 2).

Heliotrin intoxication led to an increase in the indices of endogenous intoxication: the ESR increased by 2.9 times (p1 <0.0001), the SM levels in plasma and erythrocytes increased by 2.8 times (p1 <0.0001) (Table 3). The same changes were revealed when studying oligopeptides' parameters, the content of which increased in plasma and erythrocytes by 2.8 and 2.7 times (p1 <0.0001), respectively.

Table 3

STATE OF INDICATORS OF ENDOGENIC INTOXICATION IN THE STUDY GROUPS DURING HELIOTRIN INTOXICATION AND AFTER INFUSION OF BLOOD SUBSTITUTES (M ± m)

Studied indicators	Intact, n=15	Intoxication, n=15	Intoxication + infusion:	
			The drug "Infezol-40", n=15	Amino acid blood substitute, n=15
In plasma				
SM, meth.er	12,3±0,4	34,1±0,4*	24,5±0,5*^	13,8±0,33*^#
Oligopeptides, g / l	1,3±0,04	3,6±0,03*	2,6±0,06*^	1,6±0,06*^#
Toxemia index	18,0±1,03	122,3±2,11*	63,5±2,84*^	22,5±1,55*^#
Intoxication index	28,2±1,23	197,3±3,60*	92,4±3,75*^	34,0±1,96*^#
In erythrocytes				
SM, meth.er	14,0±0,33	39,0±0,68*	26,6±0,44*^	16,8±0,31^#
Distribution coefficient, SM pl./SM er.	0,87±0,03	0,88±0,02*	0,93±0,03*	0,82±0,02*
Oligopeptides, g / l	0,7±0,02	1,9±0,03*	1,3±0,03*^	0,8±0,02*^#
Toxemia index	9,9±0,43	75,0±2,64*	34,6±1,29*^	13,6±0,53*^#
ESR , %	20,3±1,0	59,6±1,4*	33,6±1,2*^	19,5±0,9^#

Note: Designations are the same as in table 1.

Changes were also found when studying the index of toxaemia in plasma and erythrocytes, which increased by 6.8 and 7.6 times, respectively (p1 <0.0001) and the index of intoxication, which increased by 7.0 times (p1 <0.0001).

After infusion of the drug "Infezol-40" in group III, the indices of endogenous intoxication significantly decreased: ESR by 1.8 times (p2 <0.0001), SM in plasma and erythrocytes by 1.4 and 1.5 times (p2 <0.0001), the concentration of oligopeptides in plasma and erythrocytes significantly decreased by 1.4 and 1.5 times (p2 <0.0001), respectively. There was a significant decrease in the index of toxaemia in plasma and erythrocytes by 1.9 and 2.2 times (p1 <0.0001), respectively, and the index of intoxication by 2.1 times (p1 <0.0001).

As can be seen from Table 3, after the new blood substitute's infusion, the indices of endogenous intoxication also significantly decreased and were slightly lower than after the infusion of

Infezol-40, which indicates a good detoxification effect of the new blood substitute. Thus, compared with the second group, ESR decreased by 3.1 times (p2 <0.0001), SM in plasma and erythrocytes by 2.5 and 2.3 times (p2 <0.0001). The concentration of oligopeptides in plasma and erythrocytes decreased 2.3 and 2.4 times (p2 <0.0001), and the toxaemia index decreased 5.4 and 5.5 times (p2 <0.0001), respectively. The intoxication index decreased 5.8 times (p2 <0.0001).

In a comparative analysis of the effectiveness of the use of "Infezol-40" and a new blood substitute, the indices of endogenous intoxication were lower: the SM level in plasma and erythrocytes was 1.8 and 1.6 times (p3 <0.0001), the concentration of oligopeptides was 1.6 times (p3 < 0.0001), toxaemia index by 2.8 and 2.5 times (p3 <0.0001), respectively. Also, after the replacement of the new blood substitute, the ESR was significantly lower by 1.7 times (p3 <0.0001), the intoxication index by 2.7 times (p3 <0.0001), than after infusion of the reference drug "Infezfol-40".

The distribution coefficient SM pl. / SM er. was 1.1 times (p3 <0.01) less after introducing a new amino acid blood substitute.

Our data showed that heliotrin intoxication is accompanied by the development of cytolysis, cholestasis, activation of peroxidation, a decrease in the activity of the antioxidant system, and an increase in the level of endogenous intoxication. We used an amino acid blood substitute containing an antioxidant to correct these changes, which restored all of the above disorders.

CONCLUSION

1. The new amino acid blood substitute has a good detoxification effect and restores the liver's functional and metabolic parameters during heliotrin intoxication.

2. The use of a new blood substitute for heliotrin intoxication reduces the activity of hyperlipoperoxidation processes and restores AOS enzymes' activity, the general antioxidant status, showing a more pronounced antioxidant effect in comparison with the drug "Infezol-40".

Thus, in the course of the experiment, it was established that the use of a new amino acid blood substitute containing an antioxidant has a good antioxidant and detoxification effect and, as heliotrin intoxication develops, has a positive effect on the structural and functional changes in the liver caused by intoxication.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article and report on the contribution of each author.

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