STUDY OF EFFICIENCY OF APPLICATION OF A NEW INFUSION DRUG CONTAINING AMINO ACID IN EXPERIMENTAL PROTEIN DEPRIVATION

Larisa Ivanovna Shevchenko, Hamid Yakubovich Karimov, Ziyoda Bohodirovna Tolipova, Timur Raufovich Alimov, Olga Viktorovna Lubencova

Republican Specialized Scientific and Practical Medical Center for Hematology, Tashkent, Uzbekistan

Abstract. In this study, we studied the effectiveness of the new domestic blood substitute containing amino acids and the natural energy metabolite of the Krebs cycle in experimental protein starvation. **The aim** of the study was to assess the degree of influence of a new domestic amino acid preparation on metabolic homeostasis during protein starvation. **Materials and methods**. The experiments were performed on 30 male rabbits weighing 2.2-0.2 kg on a model of protein starvation. In dynamics were investigated: the state of the general antioxidant status, indicators of the blood coagulation system, hematological, biochemical parameters of blood and renal function. For comparison, the drug "Infezol-40" was used. **Results.** A study of a new domestic blood substitute containing amino acids in comparison with the Infezol 40 preparation confirmed the higher efficiency of the new domestic blood substitute in restoring the antioxidant status parameters, hemostasis parameters, blood biochemical parameters and kidney function.

***Key words**:* amino acids, homeostasis, antioxidant status, hemostasis, biochemical analysis, kidney function.

I. Introduction

Extreme conditions accompanied by deep lesions of homeostasis and protein-energy deficiency determine the need for a multicomponent correction of metabolism as part of complex intensive care [1, 2, 3, 4].

The widespread and widely used preparations of parenteral nutrition (PP) containing amino acids, including the widely used in clinical practice "Infezol-40", are not capable of complete restoration of homeostasis in severe metabolic disorders.

Developed at the Scientific Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan, a new domestic blood substitute containing amino acids and a natural energy metabolite of the Krebs cycle is able to restore various vital indicators of homeostasis [5].

II. Purpose of the research

To assess the degree of influence of a new domestic amino acid preparation on indicators of metabolic homeostasis in protein starvation.

III. Materials and methods

The protein starvation model was reproduced in 30 male rabbits weighing 2.2-0.2 kg. The animals were divided into four groups. The first group (control) consisted of intact animals that were kept on a vivarium feed: wheat flour - 12%, 9% rusks -

10%, millet - 6%, oatmeal - 36%, milk - 8%, salt mixture - 10% , fish meal - 25% feed yeast - 2.5%.

In the second group, animals were kept on a protein-free diet for 10 days. The protein-free diet included: starch and sucrose - 75%, vegetable oil - 15%, fish oil - 1%, vitamin mixture - 4%, salt mixture - 5%.

After 10 days, the experimental rabbits of the first and second groups were decapitated. The third and fourth groups of animals were kept for 10 days on a protein-free diet. Starting from the eleventh day, they were injected intraperitoneally with the studied drugs: in the third group - the drug "Infezol 40", and in the fourth - a new amino acid blood substitute at a dose of 10 ml / kg of body weight for 10 days. The drugs were administered at a dose of 10 ml / kg of body weight for 10 days, after which the animals were slaughtered in accordance with the principles set forth in the Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, France, 1986) [6].

In the dynamics in the blood plasma of rabbits, the state of the "General antioxidant status" was studied by the method of enzyme-linked immunosorbent assay (ELISA) and using the Cayman test systems (USA). The ELISA results were measured using an MR96A microplate photometer (Mindray, China).

The state of the system of the aggregation component of hemostasis was investigated on a semi-automatic analyzer of aggregation "ALAT-2" ("Biola", Russia), and the coagulation component of hemostasis - on a semi-automatic coagulometer "HumaClot Junior" (HUMAN, Germany) using reagents from HUMAN (Germany) and Renam (Russia) [7].

The study of the blood system (hematological parameters): hemoglobin, erythrocytes, MCH, MCV, MCHC, platelets, leukocytes, lymphocytes, eosinophils and reticulocytes were carried out with a hematological analyzer "ERMA INC" (Japan). To measure ESR used the standard generally accepted method of Panchenkov [8-9].

Biochemical parameters of blood were studied on a semi-automatic biochemical analyzer "BA88A" (Mindray, China) using HUMAN test systems (Germany).

Kidney function was investigated according to the generally accepted method [10].

The digital data were subjected to statistical processing using a special software package on a personal computer using Excel and Biostat programs. The criterion for statistical significance was $p < 0.05$.

IV. Results and discussion

During protein starvation, the total antioxidant status decreased by 1.5 times ($p1 < 0.01$) (Fig. 1). When studying the antioxidant status, it was found that after treatment with a new amino acid drug, it was restored to intact values or increased 1.5 times ($p2 < 0.0001$), which was 30.8% ($p3 < 0.01$) higher than after using the drug "Infezol 40" (Fig. 1).

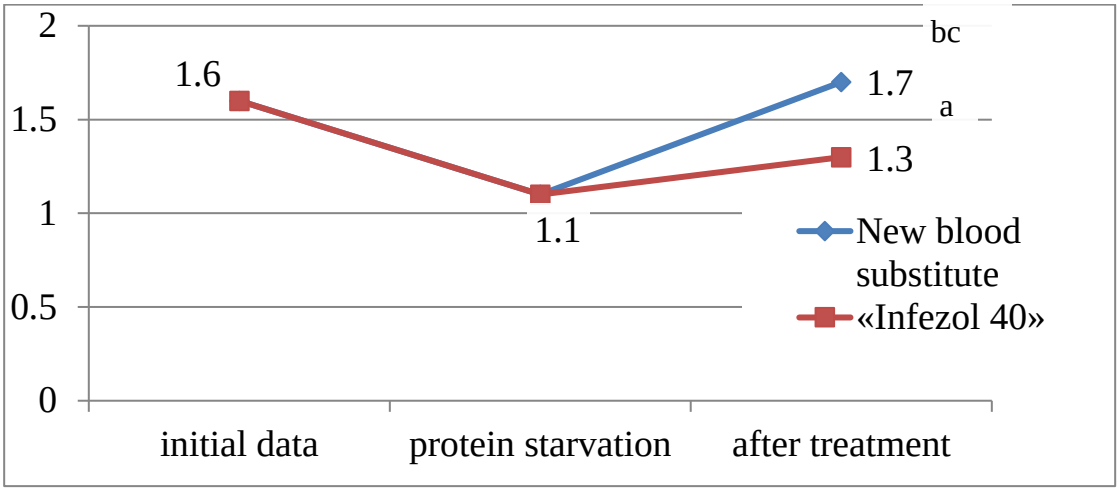

Figure 1. The state of the total antioxidant status (TAS) in rabbits during protein starvation and after the introduction of blood substitutes (M ± m) (Reference values: 0.5-2 mM)

The study of the blood coagulation system during protein starvation showed that the main indicators of the blood coagulation system were changed towards hypocoagulant shifts in the hemostatic system. Changes in hemostasis during protein starvation were also found in rabbits kept on a protein-free diet, in which APTT increased by 21.1% (p1 <0.01), PT by 28.7% (p1 <0.0001), TB by 38.8% (p1 <0.0001) and a decrease in platelet adhesion by 27.3% (p1 <0.0001), and platelet aggregation with ADP by 18.6% (p1 <0.01) (Table 1).

In rabbits, after the introduction of a new blood substitute, a decrease in APTT by 15.7% (p2 <0.02), an increase in platelet adhesion by 25.3% (p2 <0.0001) and platelet aggregation with ADP - by 13.8% (p2 <0.02), and after application of Infezol 40 there was a decrease in APTT by 12.3% (p2> 0.05), PT - by 13.2% (p2 <0.05), TB - by 17 , 8% (p2 <0.05), an increase in platelet adhesion and 24.2% (p2 <0.0001) and platelet aggregation with ADP by 10.8% (p2 <0.05) (Table 1).

Table 1

Changes in hemostasis parameters during protein starvation and after infusion of blood substitutes in rabbits (M ± m)

The studied indicators	Groups			
	I initial data, n=30	II protein starvation, n=30	III Infezol 40, n=15	V New blood substitute, n=15
APTT, sec	19,4±1,0	23,5±1,2* p_1<0,01	20,6±1,1 p_2>0,05	19,8±1,0^ p_2<0,02
PV, sec	9,4±0,3	12,1±0,6* p_1<0,0001	10,5±0,4*^ p_1< 0,05 p_2<0,05	9,6±0,9^ p_2< 0,05
TV, sec	13,3±0,8	18,5±1,2* p_1<0,0001	15,2±0,9^ p_2<0,05	14,7±0,9^ p_2<0,02

Platelet adhesion, %	49,4±1,8	35,9±1,7* p_1<0,0001	44,6±1,9^ p_2<0,0001	45,0±1,8^ p_2<0,0001
AT with ADP, %	66,5±3,2	56,5±2,0* p_1<0,01	62,6±2,1^ p_2< 0,05	64,3±2,3^ p_2<0,02

Note: * - reliability of the difference (p <0.05) when comparing the results with the initial data (group I); ^ - the same (p <0.05) when comparing the results with the data obtained during protein starvation (group II); # - the same (p <0.05) when comparing the results with the data obtained after infusion of "Infezol 40" (group III).

Thus, the protein-free diet caused changes in the parameters of the coagulation system towards hypocoagulation, and the use of a new blood substitute, like the drug "Infezol 40", contributed to their correction.

The effect of the new blood substitute on the parameters of the blood system during protein starvation was studied (Table 2).

In rabbits with protein starvation: hemoglobin was higher by 7.5% (p1 <0.01), the number of erythrocytes - by 16.7% (p1 <0.0001), as well as an increase in the number of reticulocytes by 97.1% (p1 <0.0001), as well as a slight decrease in MCH, MCV and MCHC in rabbits (Table 2). After the infusion of a new blood substitute in rabbits, comparable changes were observed: a decrease in hemoglobin concentration by 5.8% (p2 <0.01), a decrease in the number of erythrocytes by 12.7% (p2 <0.0002), and after using the drug "Infezol 40" hemoglobin decreased by 5.4% (p2 <0.05), and the content of erythrocytes - by 11.1% (p2 <0.01) (Table 2).

Table 2

Changes in blood system parameters during protein starvation and after infusion of blood substitutes in rabbits (M ± m)

Indicators	I Initial data, n=30	II Protein starvation, n=30	III «Infezol 40», n=15	V New blood substitute, n=15
Hemoglobin, g / l	124,9±2,1	134,3±2,2* p_1< 0,0002	127,1±2,0^ p_2< 0,02	126,5±2,3^ p_2<0,02
Erythrocytes, 10^{12}/л	5,4±0,2	6,3±0,3* p_1< 0,02	5,6±0,10^ p_2< 0,05	5,5±0,08^ p_2< 0,02
MCH, pg	21,1±1,1	19,6±1,0 p_1>0,05	20,4 ± 0,8 p_2>0,05	20,8±0,9 p_2>0,05
MCV, fl	65,4±3,1	62,1±2,0 p_1>0,05	63,9±2,1 p_2>0,05	64,3±2,2 p_2>0,05
MCSU, g / dl	33,0±1,2	35,8±1,3 p_1>0,05	33,7±1,2 p_2>0,05	33,1±1,1 p_2>0,05
Reticulocytes,‰	4,2±0,9	0,12±0,01* p_1< 0,0001	2,6±0,03^ p_2< 0,0001	2,9±0,02^ p_2< 0,0001

Note: * - reliability of the difference (p <0.05) when comparing the results with the initial data (group I); ^ - the same (p <0.05) when comparing the results with the data obtained during

protein starvation (group II); # - the same ($p < 0.05$) when comparing the results with the data obtained after infusion of "Infezol 40" (group III).

After the use of the new blood substitute and the reference drug "Infezol 40", as in rabbits, a tendency to recovery of such indicators as MCH, MCV and MCHC was noted.

The use of a new blood substitute and the drug "Infezol 40" also led to the restoration of the number of reticulocytes in rabbits. The infusion of the new blood substitute promoted the restoration of correction of disorders in the blood system caused by protein starvation, and the degree of recovery was at the level of the foreign analogue (the drug "Infezol 40").

Thus, the developed new amino acid blood substitute restores hemostasis and blood system parameters during protein starvation.

The results of the studies showed that during protein starvation, changes in biochemical parameters occurred in rabbits. There was a slight increase in the activity of enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), (Table 3).

Infusion of a new blood substitute and the drug "Infezol 40" led to the restoration of the values of the activity of ALT and AST, which decreased to the initial values.

In rabbits on a protein-free diet, compared with intact animals, the concentration of urea in blood plasma was 1.6 times higher ($p1 < 0.0002$), and creatinine concentration was 1.5 times higher ($p1 < 0.0001$) (Table 3).

Table 3

Changes in biochemical parameters during protein starvation and after the introduction of blood substitutes in rabbits (M ± m)

]	I Initial data, n=30	II Protein starvation, n=30	III «Infezol 40», n=15	IV New blood substitute, n=15
ALT, U / l	27,5±4,8	30,5±3,5 p_1>0,05	27,9±2,6 p_2>0,05	25,8±0,7 p_2>0,05
AST, U / l	11,4±3,1	15,1±1,2 p_1>0,05	12,5±1,4^ p_2>0,05	10,9±1,1 p_2>0,05
Urea mmol / l	8,7±0,9	13,5±1,1* p_1< 0,0002	10,6±0,5^ p_2<0,05	9,1±0,3^# p_2<0,0001 p_3< 0,02
Creatinine mmol / L	57,6±3,1	84,5±4,4* p_1<0,0001	69,3±4,0^ p_2<0,02	60,4±3,4^ p_2<0,0001
Total cholesterol, mm	2,7±0,16	1,1±0,19* p_1<0,0001	1,82±0,11^ p_2<0,01	2,15±0,14^ p_2< 0,0001
Triglycerides, mM	1,05±0,04	0,41±0,08*	0,79±0,05^	0,95±0,08^

		p_1<0,0001	p_2<0,0001	p_2<0,0001

Note* - reliability of the difference (p <0.05) when comparing the results with the initial data (group I); ^ - the same (p <0.05) when comparing the results with the data obtained during fasting; # - the same (p <0.05) when comparing the results with the data obtained after infusion of the drug "Infezol 40"

In rabbits, the infusion of a new blood substitute led to a decrease in the concentration of urea in the blood plasma by 32.6% (p2 <0.0001) and creatinine - by 28.5% (p2 <0.0001). Infusion of the drug "Infezol 40" in rabbits led to a decrease in urea content by 21.5% (p2 <0.05), creatinine by 18.0% (p2 <0.02). In a comparative aspect, after the introduction of a new blood substitute to rabbits, the concentration of urea was lower than after the use of the drug "Infezol 40" by 14.2% (p3 <0.02), and there was also a tendency to a decrease in creatinine relative to its value in III group (Table 4).

Table 4

Changes in the values of renal function indicators during protein starvation and after infusion of blood substitutes in rabbits (M ± m)

Indicators	Intact, n=15	Protein starvation №1, n=15	«Infezol 40», n=15	New blood substitute, n=15
	I group	II group	III group	IV group
GFR ml / min	14,3±0,9	11,2±0,7*	12,5±0,4^	13,8±0,8^
Diuresis, ml / min	2,4±0,18	3,1±0,18*	2,6±0,13^	2,5±0,15^

Note: * - reliability of the difference (p <0.05) when comparing the results with the initial data (group I); ^ - the same (p <0.05) when comparing the results with the data obtained during protein starvation (group II); # - the same (p <0.05) when comparing the results with the data obtained after infusion of "Infezol 40" (group III).

Thus, the use of a new preparation containing sodium succinate - an antioxidant, a natural substrate of the Krebs cycle, promoted a more effective recovery of biochemical parameters, which is consistent with the data of foreign authors [11].

Kidney function in animals during protein starvation also underwent certain changes (Table 4). In rabbits, the infusion of blood substitutes led to the normalization of renal function. The use of the new blood substitute was accompanied by the restoration of GFR by 1.2 times or by 23.2% (p2 <0.05), and after the administration of the drug "Infezol 40" there was a tendency to increase this indicator. The volume of diuresis after the infusion of the new amino acid blood substitute decreased to the values of intact animals by 1.2 times or by 19.4% (p2 <0.02), and after infusion of Infezol 40 - by 1.2 times or by 16.1 % (p2 <0.05).

Therefore, the effect of the new blood substitute containing the antioxidant sodium succinate promoted the restoration of glomerular filtration, which is consistent with the data of foreign researchers [12, 13, 14].

Thus, during protein starvation, the use of a new amino acid blood substitute helped to restore the parameters of biochemical analysis and indicators of renal function, not inferior in effectiveness to the drug "Infezol 40".

V. Conclusion

1. The new amino acid blood substitute has an antioxidant effect.

2. The new blood substitute promotes the restoration of blood parameters, hemostasis during protein starvation.

3. The use of infusion of a new blood substitute leads to the restoration of renal function and protein starvation.

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Authors:

1. Shevchenko Larisa Ivanovna - Ph.D. of the Department of molecular medicine and cell technology, Republican specialized scientific and practical medical center of hematology. 42 A, Chilanzar-6 Block, Chilanzar district, Tashkent, Uzbekistan, 100185. Correspondence author e-mail: shev.larisa@yahoo.com
2. Karimov Khamid Yakubovich – MD. Professor, Head of the Department of Molecular Genetics and Cell Technologies of the Research institute of Hematology and Blood Transfusion. 42 A, Chilanzar-6 Block, Chilanzar district, Tashkent, Uzbekistan, 100185.
3. Ziyoda Bohodirovna Tolipova – junior researcher, Research institute of Hematology and Blood Transfusion.42 A, Chilanzar-6 Block, Chilanzar district, Tashkent, Uzbekistan, 100185.
4. Timur Raufovich Alimov – Ph.D., Research institute of Hematology and Blood Transfusion.42 A, Chilanzar-6 Block, Chilanzar district, Tashkent, Uzbekistan, 100185.
5. Olga Viktorovna Lubencova – junior researcher, Research institute of Hematology and Blood Transfusion.42 A, Chilanzar-6 Block, Chilanzar district, Tashkent, Uzbekistan, 100185.