

The Physiological Effect of a New Artificial Blood on the Protein Fractions, Energy Status and the HIF-1 α Content During Protein Starvation

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Abstract In the Republican Specialized Scientific and Practical Medical Center of Hematology of the Ministry of Health of the Republic of Uzbekistan, a blood substitute containing amino acids and an antioxidant complex with a wide spectrum of action has been created. The aim of this study was to study the effectiveness of the new blood substitute on the parameters of the protein fraction, energy status and the content of HIF-1 α in the blood during protein starvation. The studies were carried out on white outbred male rats weighing 180-200 g. within 10 days. In the blood of experimental animals, the content of protein fractions and HIF-1 α , indicators of energy status were determined. The new amino acid blood substitute effectively restores blood parameters in case of protein-energy deficiency.

Keywords Blood substitute, Amino acids, Protein starvation, Na, K ATF ase, Albumin, Globulin, HIF-1 α

1. Introduction

Over the past 4 decades, based on the results of numerous studies on experimental animals and clinical observations, an unambiguous understanding was achieved the fact that most critical conditions increase the body's needs for proteins, and in individual amino acids. Maintaining protein metabolism at a high level in sick or injured people, critically ill is the most important task of intensive care. It's connected with the fact that if, for one reason or another, admission.

There are not enough nutrients, then, as we have already noted, depletion of the body occurs and hypoproteinemia and hypoalbuminemia develop. In modern conditions, in the complex of treatment, one of the leading places is occupied by infusion-transfusion therapy, which is based on the use of blood substitutes.

Despite the ability of modern blood substitutes to correct various vital parameters of homeostasis in general, none of the existing infusion preparations was able to restore the function and viability of cells.

In this regard, a new amino acid blood substitute was created at the Republican Specialized Scientific and Practical Medical Center of Hematology (RSNPMTSG) of the Ministry of Health and antioxidant action, mannitol and sorbitol-having osmotic, detoxifying action.

The purpose of this section is to study the effectiveness of the action of the new amino acid blood substitute on the indicators of protein-energy metabolism and the content of HIF-1 α in the blood during protein starvation in the experiment.

2. Materials and Methods

White outbred male rats weighing 180-200 g (n = 120) were used as experimental animals.

The animals were divided into four groups. The first group consisted of intact animals that were kept on a vivary feed: wheat flour - 12%, 9% rusk - 10%, millet - 6%, oatmeal - 36%, milk - 8%, salt mixture - 10%, fish meal - 25% feed yeast - 2.5%.

In the second group, the 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%.

Group III (control, comparison) - with an experimental model of protein-energy malnutrition after administration of the amino acid comparison drug Infezol 40. Animals of the third group will contain animals for 10 days on a protein-free diet. Starting from the eleventh day, they are injected intraperitoneally with the drug "Infezol 40" at a dose of 10 ml / kg.

Group IV (main, experimental) - animals with an experimental model of protein-energy deficiency after the

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introduction of a new amino acid blood substitute. The fourth group will contain animals for 10 days on a protein-free diet. Starting from the eleventh day, they are injected intraperitoneally with a new amino acid blood substitute at a dose of 10 ml / kg of body weight for 10 days.

To assess the degree of imbalance of anabolic and catabolic processes caused by protein starvation, the content of total protein, albumin, was determined. Biochemical blood parameters were studied on a BA88A semi-automatic biochemical analyzer (Mindray, China) using HUMAN test systems (Germany) [22]. Protein fractions were also determined by the turbidimetric method according to the generally accepted method.

The ATP content in the blood of experimental animals was investigated by the biochemical method of research using test systems (Boehringer, Mannheim, Germany). The measurements were performed on a UNICO 2800 spectrophotometer (United Products and Instrument, Inc., USA). ATPase indices were performed according to the method of Kazzenov *et al.* [8]. Preliminarily separated from plasma and washed 3 times with a buffer containing 0.145 mM NaCl, 10 mM Tris-HCl, (pH 7.4), erythrocytes were treated with 1% tween-20 in 0.25 M sucrose in Tris-HCl buffer (volume 1: 1, exposure 60 min at 20°C). The Na⁺, K⁺ -ATPase activity was calculated from the increase in inorganic phosphorus (Fn, sensitivity 0.01 μ M) in the incubation medium and was calculated from the difference between the ATPase activity without an inhibitor in the presence of an inhibitor - 1 mM ouabain. The activity was expressed in μ mol Fn in h per ml of cells (taking into account the hematocrit of the prepared suspension of erythrocytes). The following reagents were used: EDTA, ATP and ouabain (Sigma) and other reagents (Russia).

The content of blood electrolytes (sodium, potassium, chlorine) was determined by biochemical analysis using Human test kits (Germany). Measurements were performed

on a BA88A semi-automatic biochemical analyzer (Mindray, China).

The concentration of HIF-1 α was determined by the enzyme-linked immunosorbent assay (ELISA) using the Cloud-Clone test systems (Cloud-Clone corp., USA). Measurements were performed at a wavelength of 450 nm using an MR96 microplate photometer (Mindray, China). The results are expressed in ng / ml.

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

3. Result and Discussion

The content of total protein in blood plasma in rats that did not receive blood substitutes during experimental protein starvation in group II decreased by 22.7% ($p_1 < 0.01$) (Table 1).

The study of protein fractions during protein starvation showed that when rats were kept on a protein-free diet for 10 days, the albumin content decreased by about 21.2% ($p < 0.01$), and the use of a new blood substitute increased the albumin content in the blood by 28, 6% ($p < 0.05$), compared with group II. Administration of Infezol 40 resulted in approximately the same increase in the albumin content in the blood plasma of rats by 19.4% ($p < 0.05$).

The introduction of a new blood substitute in group IV in rats led to an increase in the concentration of total protein by 24.8% ($p_2 < 0.01$), and the use of Infezol 40 in group III - by 16.8% ($p_2 < 0.01$) ... At the same time, the introduction of a new blood substitute and the drug "Infezol 40" led to the restoration of the total protein to the initial values, which indicates the effectiveness of the action of the new blood substitute on protein metabolism, which is not inferior to the foreign analogue.

Table 1. Changes in the fraction of blood plasma proteins during protein starvation and after the use of blood substitutes in rats ($M \pm m$)

Indicators	Initial data, n=30	Protein starvation, n=26	Infezol 40, n=28	New blood substitute, n=30
	I group	II group	III group	IV group
Total protein, g / l	72,6 \pm 0,67	56,1 \pm 0,70	65,5 \pm 0,83	70,0 \pm 0,74
Albumin fraction I, g / l	28,8 \pm 1,5	22,7 \pm 0,3	27,1 \pm 0,33	29,2 \pm 0,3
Globulins:	43,8 \pm 0,41	33,4 \pm 0,42	38,7 \pm 0,47	40,8 \pm 0,43
Fraction 2 alpha1, g / l	2,0 \pm 0,2	1,4 \pm 0,02* p 0,02	1,7 \pm 0,08	1,8 \pm 0,02
Fraction 3 alpha2, g / l	9,1 \pm 0,8	4,6 \pm 0,06* p1<0,0001	6,4 \pm 0,08^ p<0,0001	6,7 \pm 0,07^ p<0,0001
Fraction 4 beta, g / l	16,7 \pm 0,8	8,5 \pm 0,1* p<0,0001	10,7 \pm 0,1^ p<0,0001	11,6 \pm 0,1^ p<0,0001
Fraction 5 gamma, g / l	23,4 \pm 1,6	16,6 \pm 0,2* p<0,0001	19,9 \pm 0,2^ p<0,0001	20,7 \pm 0,22^ p<0,0001
A/G	0,66 \pm 0,00	0,68 \pm 0,00	0,70 \pm 0,00	0,71 \pm 0,00

Table 2. Changes in energy parameters in blood plasma during protein starvation and infusion of blood substitutes in rats ($M \pm m$)

Indicators	Initial data, n=30	Protein starvation, n=26	Infezol 40, n=28	New blood substitute, n=30
	I group	II group	III group	IV group
ATP, conv. units	6,48±0,23	5,02±0,07* p<0,05	6,85±0,09^ p<0,001	8,02±0,11^# p<0,0001, p<0,05
Na +, K + -ATPase, μmolFn / h / ml	16,2±0.15	10,6±0,1* p<0,001	14,1±0.15^ p<0,05	16,7±0,2^ p<0,001
Mg2 + -ATPase, μmolFn / h / ml	27,1±0.3	19,6±0,3* p<0,01	22,8±0,28 p>0,05	27,1±0,3^# p<0,001, p3<0,05
Na + plasma, mM	141,4±0.3	137,9±0,9* p<0,05	140,8±0.9^ p>0,05	141,3±0,7^ p<0,05
K + plasma, mM	5,33±0,12	4,11±0,04* p<0,001	5,20±0,05^ p<0,001	5,29±0,05 p<0,001

Note: the same as in table 1.

Table 3. Changes in the content of hypoxia-inducible factor (HIF-1α) for protein-energy malnutrition (up to 0 mm Hg)

Index	Intact, n=30	control, n=26	After treatment:	
			preparation "Infezol 40" n=28	New amino acid blood substitute, n=30
	I group	II group	III group	IV group
HIF-1α, ng/ml	0,07±0,002	0,11±0,004, p1<0,01	0,09±0,003, p1<0,01, p2=0,01	0,08±0,003, p2<0,001, p3<0,001

Note: * - p < 0.05 compared with group I; ^ - p < 0.05 compared with the second group; # - p < 0.05 compared with group III
p-criterion of reliability (p < 0.05) p1 - relative to the first group; p2 - relative to the second group; p3 - relative to the third group

After the application of the new blood substitute in group IV, the rats showed the most significant increase in the content of the 4th and 5th fractions of globulins by 36.5% and 24.7% (p < 0.01), respectively (Table 1), and after the introduction of the drug "Infezol 40" - by 25.9% and 19.9%, respectively.

The 2nd and 3rd fractions of globulins do not undergo significant changes in all cases within 10 days of using the drugs, and the content of the 3rd fraction is even slightly, statistically insignificantly reduced. Proteins of the 4th and 5th fractions are synthesized more actively than in the 2nd and 3rd fractions, but their content at the end of the course of using the blood substitute does not exceed the total level of globulins. In general, the recovery of globulins is slower than that of albumin. After the use of the new blood substitute, the values of the globulin fractions were at a comparable level with the results after the use of Infezol 40 in group III.

Thus, the results of the study of the effectiveness of the new blood substitute on the protein starvation model showed that the new blood substitute restores protein, energy metabolism and electrolyte balance. As can be seen from table 1, a comparison of the results of the study of the effect of the new blood substitute with the effect of the use of "Infezol 40" showed that they approximately equally restored body weight, protein metabolism and electrolyte balance within 10 days.

The effect of the new blood substitute on the parameters of energy metabolism during protein starvation in rats was studied, for example, in group II, the value of ATP content

decreased by 22.5%, relative to group I (p < 0.05) (Table 2). The study of changes in the activity of Na +, K + -ATPase showed that protein starvation leads to a decrease in its values in group II by 34.6% (p1 < 0.02), and the infusion of a new blood substitute contributed to an increase in its activity, after the introduction of which in group IV the activity Na +, K + -ATPase increased, relative to its value during protein starvation in group II by 57.5% (p2 < 0.001).

After the use of the new blood substitute in group IV, an increase in the ATP content in the blood of experimental individuals was observed by 59.8% (p < 0.001). The introduction of the drug "Infezol 40" in group III led to the restoration of ATP and its increase by 36.5% (p2 < 0.01) (Table 2). In a comparative study of the results obtained after the use of a new blood substitute in group IV, compared with the results after the drug "Infezol 40" in group III, the ATP content in the blood was 17.1% higher (p3 < 0.05). Also, during protein starvation, the activity of Mg2 + -ATPase decreased by 27.7% (p1 < 0.01), and the use of a new blood substitute restored it to its initial values - by 38.3% (p2 < 0.01). No such recovery was observed after the administration of Infezol 40 (Table 3).

The study of the concentration of electrolytes during protein starvation showed that the content of sodium and potassium ions after the introduction of a new blood substitute and the drug "Infezol 40" was restored to the same extent.

Thus, during protein starvation, it was possible to observe a decrease in the quantitative values of the main parameters characterizing protein and energy metabolism, and a

decrease in the values of the electrolyte balance parameters was observed. The use of the new blood substitute led to approximately the same restoration of indicators of protein metabolism and electrolyte balance.

However, when using the new blood substitute, the energy metabolism indicators were restored to a greater extent, which was not observed after the use of the "Infezol 40" drug. This is due to the introduction of an antioxidant complex with energy activity into the new blood substitute, which is able to normalize not only protein, plastic metabolism and electrolyte balance, but also actively restore energy metabolism in the cell.

On the model of protein starvation in rats, significant changes in the content of hypoxia-inducible factor (HIF-1 α) in the blood were revealed (Table 3). In case of protein-energy deficiency, HIF-1 α increases by 57.1%. After the introduction of the new blood substitute HIF-1 α , its decrease to the initial values is observed, which is not observed after the use of the drug "Infezol 40" ($p < 0.05$).

4. Conclusions

In a comparative study of the new amino acid blood substitute, it was proved that it restores the indicators of protein fractions and energy status, also has an antihypoxic effect in protein-energy deficiency and can be used in medical practice, in pathological conditions associated with protein-energy deficiency.

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