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COMPARATIVE EVALUATION OF THE EFFECTIVENESS OF TREATMENT METHODS FOR RATED PHENYL HYDRAZINE-HEMOLYTIC ANEMIA IN RATS

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Abstract

The aim of the study was a comparative assessment of the effectiveness of the new Reomannisol blood substitute on the state of peripheral blood and bone marrow parameters in experimental hemolytic anemia.

Materials and methods. Studies were conducted on 120 outbred white sexually mature male rats. Of the total number of rats, 100 animals were injected with a 2% solution of phenylhydrazine at a dose of 50 mg / kg of animal weight once intraperitoneally to simulate hemolytic anemia (one day). Then, on day 1 of the experiment, the studied animals (n = 120) were randomly divided into five groups, depending on the treatment. In all groups of animals, the dynamics of hemogram indicators was studied.

Results. Analysis of the dynamics of hemogram indices in rats with phenylhydrazine hemolytic anemia during treatment showed the high efficiency of the new domestic blood substitute Reomannisol, which contributed to a significant restoration of the reactivity and functional reserves of erythron in rats, which resulted in an increase in the level of hemoglobin, erythrocytes and reticulocytes.

Keywords: Hemolytic anemia, phenylhydrazine, hemogram indices, treatment.

Introduction

Anemia is one of the biggest complications in the health care system that is prevalent around the world. Regardless of its categories and cause, it is characterized by a decrease in the number of hemoglobin and red blood cells. It

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causes the process of hypoxia to occur in the body, and thus the condition of anemia can be the cause of serious life-threatening complications [11]. Among the various forms of a large group of anemia, hemolytic anemia is a common class of anemia caused by many exogenous and endogenous factors [9,10]. Of important clinical importance is the disruption of the mechanisms that ensure the natural tolerance of erythroid blood cells in hemolytic anemia (HA), and that this disruption leads to hemolysis processes. Therefore, an in-depth study of these mechanisms is considered necessary.

One of the most pathogenetically rational experimental models reflecting the observed changes in anemia can be considered to be the model of phenylidrazine intoxication [7,8]. Hypoxia, a characteristic sign of anemia, a decrease in the aerobic state of the blood, minimal influence on biochemical processes, adequately reflects the processes occurring in anemia, which makes it possible to apply in the study of pathology [6].

Of particular importance are studies to assess the status of peripheral blood parameters and myelograms in experimental HA. Damage and destruction of erythrocytes leads to the activation of various pathological processes in the body, which leads to the formation of hypoxia, failure of many organs and death. In such cases, it is necessary to urgently transfuse the donor's blood into the body, but this is not always possible in practical terms. Therefore, the search for alternative methods of blood transfusion replacement in the treatment of hemolytic anemia remains a relevant scientific direction today [1,5].

The Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan has developed a new local drug with antioxidant and diuretic properties, containing sodium succinate (succinate acid), mannitol and electrolytes near blood plasma [2, 3, 4]. In this regard, a new drug "Reomannisol", which allows to positively influence metabolic processes, reduce the level of intoxication, restore the impaired acid-alkali balance and electrolyte metabolism, is recommended as a promising means to solve this problem.

The aim of the study was to comparative assessment of the morphological parameters of peripheral blood status and bone marrow in experimental hemolytic anemia.

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Materials and methods. Studies were conducted on 120 non-pedigree sexualized white male rats. Given that one dose of 50 mg/kg (daily) weight of animals per 100 animals was administered intraperitoneally intraperitoneally 2% phenyligrazine solution. Then, on day 1 of the experiment, the studied animals (n = 120) were randomly divided into groups of five depending on treatment.

Group I (n = 20) - undiseased animals;

Group II (control, n = 25) – with the HA experimental model;

Group III (control, comparisons, n = 25) - with an experimental model of GA after a physiological solution (sodium chloride solution 0.9%);

Group IV (basic, comparisons, n = 25) – animals presented with an experimental model of hemolytic anemia after treatment with rheosorbilact;

Group V (control, experimental, n = 25) - animals presented with an experimental model of hemolytic anemia after treatment with the new blood replacer Reomannisol.

Sodium Chloride Solution 0.9%, Rheosorbilact and Reomannisol were administered for 5 days at a dose of 10 ml / kg of animal weight, the day following the administration of phenylhydrazine.

Part of the scientific experiment was conducted at Vivarium of the Tashkent Medical Academy.

On days 1, 2 and 5 of the experiment, experimental animals underwent beheading under mild anesthesia. Blood (1-2 ml) with EDTA was collected from the caudal vein into separate tubes with EDTA to study total blood count indicators (determination hemoglobin concentration, red blood count, color index, platelet, leukocytes, erythrocyte precipitation rate) using a hematology analyzer Mindray (China). Laboratory studies were performed in the scientific laboratory of the Department of Molecular Medicine and Cell Technologies of the Research Institute of Hematology and blood transfusion (Tashkent).

The obtained data were carried out using Microsoft Office Excel programs according to the criteria of requirements for statistical processing, with the help of which the mean value (M), standard deviation (sigma (σ)) and standard deviation (m) and standard error (m) and "Biostatistics 4.03" were calculated, with the help of which t and p-criteria were calculated. The statistical significance criterion was $p<0.05$.

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Results of the study

Phenylidrazine-induced use resulted in the development of hemolytic anemia in experimental animals, as evidenced by changes in hematologic parameters on hemogram and myelogram (Table 1). In particular, on the 1st day of the experiment, a significant decrease in the level of hemoglobin and erythrocytes was observed in group II: 102.6±3.5 g / l (pI<0.05) and 2.6±0.11 x 1012 / ml (pI<0.001) 123.0±7.4 g / l and 123.0±7.4 X 1012 / ml. On day 2, the content of hemoglobin and red blood cells in group II decreased to 94.1±3.8 g / l (pI<0.01) and 2.0±0.09 x 1012 / ml (pI<0.001). The fact of the largest decrease in hemoglobin and erythrocytes was established on the 5th day of the experiment, when the values of 69.5±2.8 g / l (pI<0.001) and 1.65±0.09 x 1012 / ml) (pI<0,001) were lower than in the undisturbed group. The changes identified are a direct result of hemolysis of erythrocytes under the action of CBC-made phenylhydrazine, confirming the fact of the development of hemolytic anemia with hyperchromic character (Table 1).

Table 1 Change of haematological indicators during experimental hemolytic anemia

CBC indicators	Igr-unbroken, n = 10	IIgr-HA, n=25		
		1-day	2- day	5- day
Erythrocyte	3,6±0,16	2,6±0,11 ***	2,0±0,09 ***	1,65±0,09 ***
Hemoglobin	123,0±7,4	102,6±3,5*	94,1±3,8 **	69,5±2,8 ***
Color Index	1,02±0,03	1,06±0,04	1,09±0,04	1,3±0,05 ***
Reticulocytes	6,6±0,64	12,3±0,55	16,7±0,77 ***	26,80±0,92 ***
Platelets	230,0±15,82	220,8±9,3	194,5±8,9 *	169,8±8,3 **
Leukocyte	6,7±0,45	6,2±0,30	6,26±0,30	6,30±0,33
ESR	5,0 ±0,68	9,0 ±0,53	11,0 ±0,58 ***	18,0 ±0,87 ***

Note: Reliability (p<0.05) compared to animals without pI contamination (group I).

In the second group of animals, the physiological reaction of the bone marrow to a sharp decrease in the oxygen transport function of the blood associated with the accelerated destruction of erythrocytes increased the proportion of reticulocytes by

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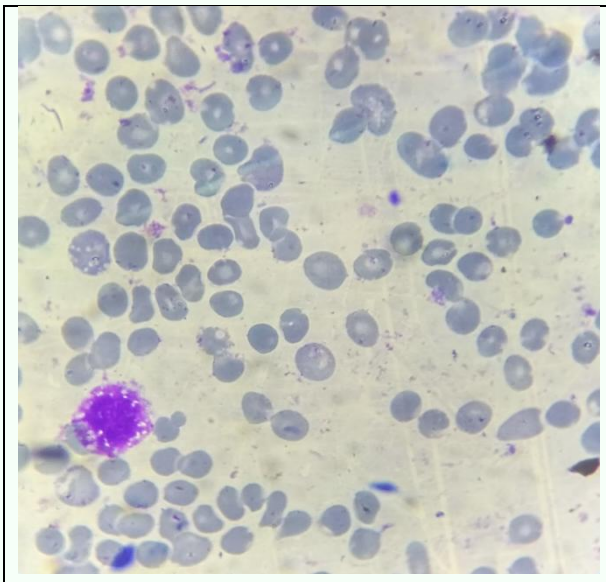
1.9 times (12.3±0.55% on day 1; in the undisturbed group, by 6.6±0.64%), by 2.5-fold (16.7±0.77%; pI<0.001), and by 4.06-fold (26.80±0.92% pI<0.001) on Day 5. In terms of the number of platelets and leukocytes, a slight decrease was noted in the group of animals with the hemolytic anemia model, possibly due to the toxic effect of phenylhydrazine, but at the same time their values did not differ significantly from those in the non-impaired group.

Along with the above changes, erythrocyte precipitation rate (ESR) was determined, in particular, on Day 1 of the experiment its level increased to 9.0±0.53 mm/hr (5.0±0.68 mm/hr in the intact group), on Day 2 this indicator was 11.0±0.58 mm/hr, on Day 5 - 18.0±0.87 mm/s The ESR level on days II and 5 increased significantly from 2.2 (pI<0.001) and 3.6 (pI<0.001) times (Table 1) to the undisturbed group, This may indicate a decrease in the cell-plasma ratio in the blood and changes in the electrical potential of red blood cell membranes due to hemolysis. The study of the morphology of blood red blood cells in animals of the second group made it possible to determine the presence of anisocytoses of poicyolocytosaerythrocytes, which were especially detected on the 5th day of the experiment. In particular, in relation to normicides, an increase in the content of micro- and macroerythrocytes in the blood was observed (Fig. 1).



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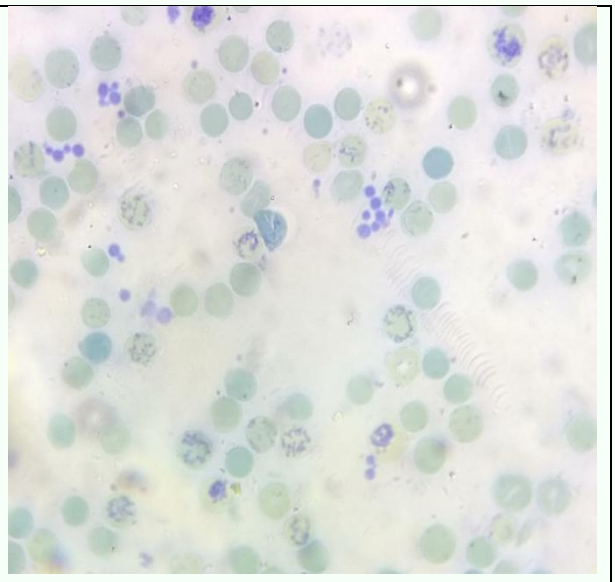

Б

Figure 1. Morphological image of blood in rats with phenylidrazine hemolytic anemia. poikilocytosis of α-anisotcytosis and erythrocytes; B-reticocytosis

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In addition, we studied the dynamics of hemogram parameters under the treatment effect, during which significant differences were found depending on the drug from which CBC was performed. Thus, on the 1st day of the experiment, the values of hemoglobin and erythrocytes in the III experimental group injected with physiological solution were recorded at 102.8±3.2 g/l (pI<0.05) and 2.90±0.16 x 1012/ml (pI<0.01). On day 2, the hemoglobin and erythrocyte levels were 95.8±3.0 g/l (pI<0.01) and 2.61±0.13 x 1012 / ml (pI<0.001). On day 5, the hemoglobin concentration and erythrocyte count were 96.2±2.8 g/l (pI<0.001) and 2.72±0.15 x was 1012/mL (pI<0.001). At the same time, the reticulocyte content in this group was 14.40±0.71% (pI<0.001) on Day 1, 17.2±0.90% (pI<0.01) and 17.8±1.02% (pI<0.001) on Day 1; pII<0.05)(Table 2.).

Table 2 Dynamics of hematological parameters in animals treated with a physiological solution

CBC indicators	IIIgr-HA, n=25		
	1-day	2-day	5-day
Erythrocyte	2,90±0,16 **^^^	2,61±0,13 ***	2,72±0,15 ***
Hemoglobin	102,8±3,2 *	95,8±3,0 **	96,2±2,8 **
Color Index	1,07±0,05	1,01±0,05	1,06±0,05
Reticulocytes	14,40±0,71 ***^	17,2±0,90 ***	17,8±1,02 ***
Platelets	210,2±11,2	196,0±9,6	180,0±9,1 *
Leukocyte	6,18±0,34	6,25±0,36	6,24±0,47
ESR	9,00 ±0,65 ***	11,00 ±0,65 ***	10,0 ±0,61 ***^^^&&

Note: *- Significantly compared to Group I indicators (*-P<0.05; **-P<0.01; ***-P<0.001); ^- Significantly compared to Group II indicators (^-P<0.05; ^^-P<0.01; ^^^-P<0.001); &- Compared with Group III indicators (&-P<0.05; &&-P<0.01; &&&-P<0.001)

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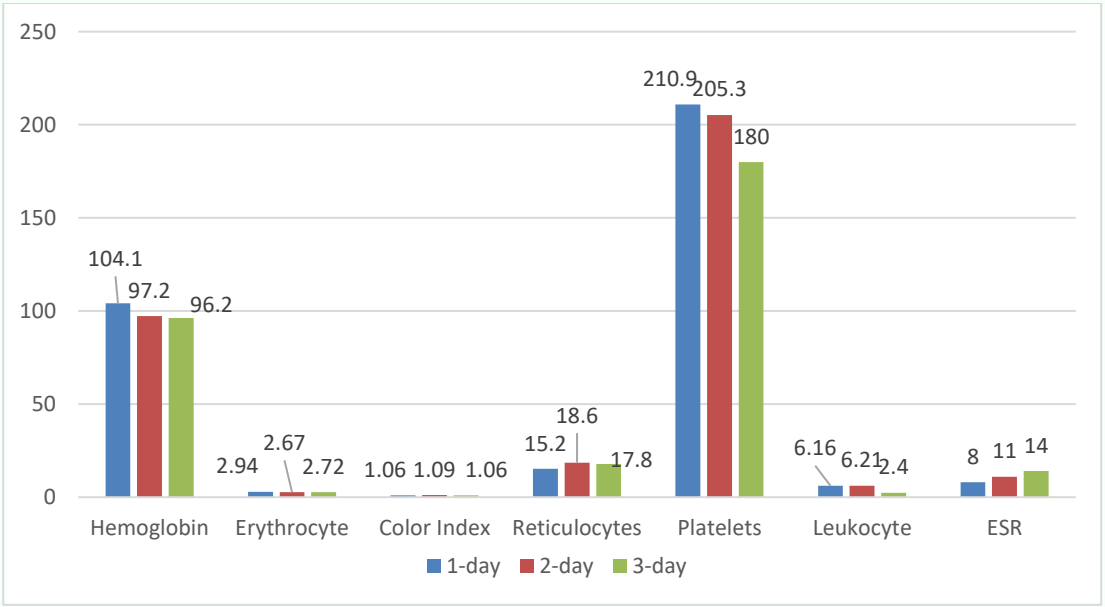
As in the animals of the second group, the number of platelets and leukocytes did not deviate from the norm. The level of ESR on days 1 and 2 practically did not differ from that in the second experimental group, but on day 5 this parameter decreased by 14.0 ±1.1 mm/hr (pI<0.001; pII<0.05).

The morphological picture of erythrocytes in this group is characterized by the presence of anisocytosis and poikilocytosis.

Thus, the data stored in rats with HA against the background of treatment with a physiological solution (group III) indicate that phenylidrazine is less effective in the treatment of HA (group II) than the data on hemogram parameters treated with (group II).

Analysis of hemogram data in group IV treated with rheosorbilact: hemoglobin concentration, erythrocyte and reticulocyte count up to 104.1±3.9 g/l(pI<0.05) on the 1st day of experiment;2.94±0.0 12 x 1012/ml (pI<0.01) and 15.20±0.61% (pI<0.001; pII<0.01), on Day 2 97.2±4.2 g/L (pI<0.01); 2.67±0.10 x 1012/ml (pI<0.001) and 18.60±0.97% (pI<0.001) on Day 5 99.8±3.4 g/L (pI<0.001); 2.78±0.13 x 1012/ml (pI<0.05) and 19.67±1.1% (pI<0.001) (Fig. 2).



Group. 2. Dynamics of hemogram parameters in IV group of animals

At the same time, the level of platelets and leukocytes remained at normal values. The ESR index was slightly lower on Day 1 ±0.46 mm/hr (pI<0.01), on Day 2 it was 10.0±0.66 mm/s (pI<0.001) and on Day 5 it was 10.0±0.61 mm/s (pI<0.001; pII<0.001; pIII<0.01).

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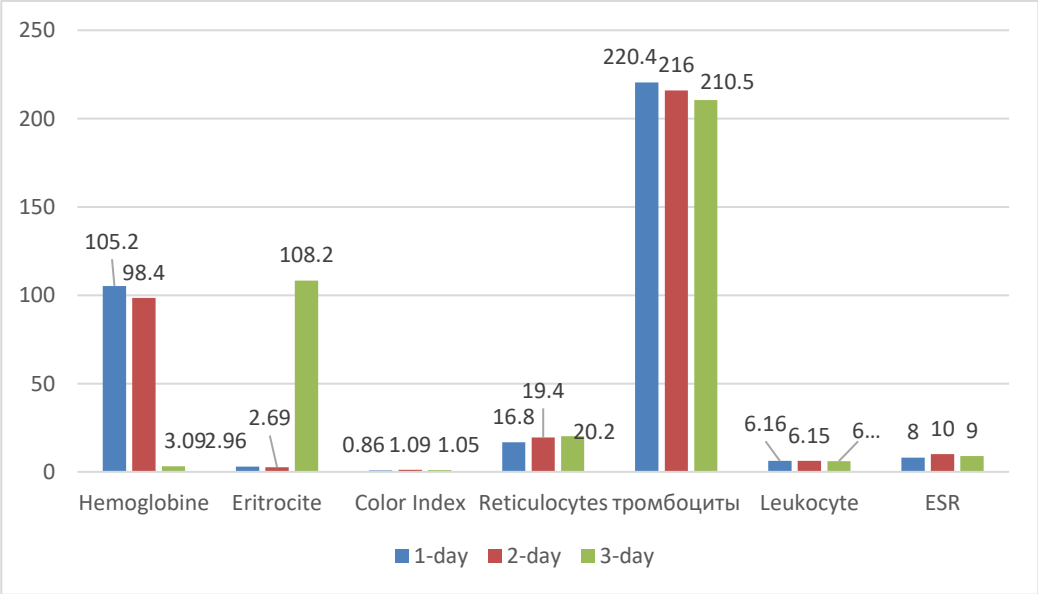
With these changes, the morphological parameters of erythrocytes differed from those in groups II and III with a decrease in the level of anisocytosis and poikilocytosis on day 5 of the experiment.

Thus, in the study of hemogram parameters against the background of treatment with rheosorbilact in animals with HA (group IV), a positive dynamics was noted in animals treated with a physiological solution (group III).

Studies in group V of animals treated with the new internal blood replacer Reomannisol showed a significant difference in the dynamics of hemogram parameters in comparison with the previous experimental groups. In particular, the hemoglobin level, erythrocyte and reticulocyte count rose to 105.2±4.2 g/l(pI<0.01) on the 1st day of the experiment;2.96±0.10 x 1012/ml (pI<0.05) and 16.05 8±1.2% (pI<0.001; pII<0.01), 98.4±3.7 g/l (pI<0.001) on Day 2); 2.69±0.11 x 1012/ml (pI<0.01) and 19.4±1.3% (pI<0.001) and Day 5 108.2±3.2 g/l (pI<0.001); 3.09±0.12 x 1012/ml (pI<0.01) and 20.2±1.2% (pI<0.001). Day 1 (8.0±0.46 mm/hr; pI<0.01) and Day 2 (8.00.46 mm/hr; pI0.01) and Day 2 The ESR level on day (10.0±0.66; pI<0.001) was nearly the same as in the rheosorbilact-treated animal group, 9.0±0.46 mm/hr (pI<0.001; pII<0.001; pIII<0.01) on day 5 compared to 10.0±0.61 mm/hr in group IV (Fig. 3).

In addition, the degree of anisocytosis and poicyolocytosis of erythrocytes on the 5th day of the experiment was maximized by positive dynamics in comparison with the dynamics in the studied groups.



Picture. 3. Dynamics of hemogram parameters of animals of group V

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Thus, the evaluation of the dynamics of hemogram parameters in mice with phenyldrazine hemolytic anemia against the background of treatment with the drug Reomannisol a novel local blood replacer should be noted that at the end of follow-up (day 5), most of the quantitative indicators and quality characteristics of the hemogram returned to the level of the intact group. The obtained data indicate a high efficacy of this drug, which helps to significantly restore the reactivity and functional reserves of erythron in rats, resulting in an increase in the level of hemoglobin, erythrocytes and reticulocytes.

Conclusions:

1. Hemolytic anemia modeled with phenylidrazine is characterized by decreased hemoglobin levels, decreased red blood cell count, development of anisocytosis and poicylkytosis, increased blood reticulocyte count, and increased erythrocyte precipitation rate (ESR).
2. In the case of anemia caused by phenylhydrazine, the use of the new local drug Reomannisol is characterized by a significant restoration of the hemoglobin and erythrocyte count. This drug stimulates erythropoiesis, provokes the occurrence of compensatory reticulocytosis, and also has a positive clinical effect with a decrease in the level of anisotsitosis and poicilocytosis.

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