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EFFICIENCY OF A NEW INFUSION DRUG WITH ANTIOXIDANT AND ANTIHYPOXANT EFFECT IN ACUTE BLOOD LOSS

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Abstract

In the Republican Specialized Scientific and Practical Medical Center of Hematology (RSNPMTSG) of the Ministry of Health of the Republic of Uzbekistan (formerly the Scientific Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan) together with the Institute of Chemistry of Plant Substances named after academician Yunusov S.Yu (ICPS) has developed a new infusion preparation of hemodynamic, antioxidant and antihypoxant action, containing a complex of polysaccharides and a metabolite of the Krebs cycle.

The work aims to compare the hemodynamic and antioxidant properties of the drug "Rheoambrasol" in acute hemorrhage, with "Rheopolyglukin" widely used in medical practice.

The work was performed on a model of acute blood loss in 40 rabbits. Hypoxia-inducible factor (HIF-1a), as well as hemodynamic parameters, as well as lipid peroxidation (LPO), and the state of the antioxidant system (AOS) were studied.

The use of a new drug in blood loss leads to the restoration of hemodynamics, inhibition of lipid peroxidation (LPO), and stabilization of the antioxidant system (AOS).

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抽象的
在乌兹别克斯坦共和国卫生部（原乌兹别克斯坦共和国卫生部血液学和输血科学研究所）共和国血液学专业科学与实用医学中心（RSNPMTSG）与该研究所合作以尤努索夫 S.Yu 院士命名的植物物质化学专业 (ICPS) 开发了一种具有血液动力学、抗氧化和抗低氧作用的新型输液制剂，含有多糖和克雷布斯循环代谢物的复合物。
该工作旨在比较药物“Rheoambrasol”在急性出血中的血液动力学和抗氧化特性，以及广泛用于医疗实践的“Rheopolyglukin”。
这项工作是在 40 只兔子的急性失血模型上进行的。研究了缺氧诱导因子 (HIF-1a)、血流动力学参数、脂质过氧化 (LPO) 和抗氧化系统 (AOS) 的状态。
在失血中使用新药可恢复血流动力学、抑制脂质过氧化 (LPO) 和稳定抗氧化系统 (AOS)。
关键词：多糖，克雷布斯循环代谢物，失血，缺氧诱导因子（HIF-1α），脂质过氧化（LPO），抗氧化系统（AOS）。

Introduction

The problem of creating drugs with hemodynamic effects remains relevant. Experience with these tools showed a number of significant shortcomings associated primarily with the ability to adversely affect the adhesive-aggregation characteristics of blood cells and the hemostasis system and the development, although rarely, of anaphylactogenic type reactions (1,2).

It is in this range that it is of interest to include into the composition of infusion corrector polysaccharides of plant origin, which retain optimal biological and hemodynamic properties inherent in the known hemodynamic blood substitutes of this series and do not affect the hemostasis system. In combination with the energy substrate, the polysaccharide is able to restore cell metabolism, stabilize disorders of metabolic acidosis and water-electrolyte balance. Besides, polysaccharides of plant origin Gledicia are non-toxic and have cytoprotective properties. Plants of the genus Gledicia belong to this. Fabaceae and Gledicia are rich in various beneficial substances, and their fruits have long been used in folk medicine in Eastern countries. Alkaloids, flavonoids, pigments, antra-glycosides, and tannins were found in them. The young leaves of the common blood locust (G. triacanthos) contain 1% of alkaloid - triacanitine, which has an antispasmodic effect [1], caffeic acid, and cyanidin are also found in the leaves [2]. The plant's flowers contain 0.3% alkaloids, 100-400 mg /% ascorbic acid in leaves and fruits, saponins in the pericarp [3], and 2-6% antra-glycosides, 3.1% tannins in the leaflets of the beans, and traces of vitamin K [4]. In aqueous solutions, water-soluble polysaccharides – galactomannans were obtained. Galactomannans are markers of this genus of plant. These polysaccharides have antibiotic, antiviral, antitumor, antidote activity. In addition, they contribute to the elimination of toxic substances from the body, cholesterol, heavy metals, radionucleotides and prevents the formation of

free radicals, restore damaged cells, as well as anti-radiation agent and activate the immune system.

So, at the Republican specialized scientific-research medical center of hematology (RSSPMCH) of the Ministry of Health of the Republic of Uzbekistan (), together with the Institute of Plant Chemistry (IPC) of the Academy of Sciences of the Republic of Uzbekistan, a polyfunctional action new infusion corrector was created using polysaccharide galactomannan and a bioenergy substrate, conditionally named "Rheoambrasol" (3,4,5)

The last question should lead to your purpose statement.

The work aims to compare the hemodynamic and antioxidant properties of the drug "Rheoambrasol" in acute blood loss, with the widely used in medical practice drug "Rheopolyglukin."

Materials And Methods

The work was performed on a model of acute blood loss in 40 rabbits by males weighing 2.2 ± 0.2 kg. The animals were divided into four groups: 1 - intact, 2 - after blood loss, 3 - after blood loss and infusion of the drug "Rheopolyglukin," 4 - after blood loss and input of a new infusion corrector with hemodynamic action.

Acute blood loss was modeled by fractional bleeding from the femoral artery for one hour until blood pressure was reduced to 40 mm Hg. Simultaneously, in the first 15 minutes of blood loss was 1/3 of the animal's body weight. The total amount of blood released into the reservoir corresponded to 27.0 ± 2.6 ml/kg. One hour after blood loss, the animals were once infused with a new blood substitute containing a polysaccharide in a volume of 40 ml per 1 kg of body weight, which is 1.6 ± 0.1 blood loss.

Hemodynamic parameters were studied over time - blood pressure (BP) was measured manometrically in the femoral artery, the minute volume of blood circulation (MVBC) was determined by thermodilution, the circulating blood volume (CBV) was determined by dilution of Evans blue and calculated using the generally accepted formula. Central venous pressure (CVP), total peripheral vascular resistance (TPVR), and systemic oxygen transport (SOT) were calculated by the generally accepted formulas. We also investigated lipid peroxidation (LPO), malonic dialdehyde (MDA), diene conjugates (DC) and diene ketones (DK), the state of the antioxidant system (AOS) (catalase, superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPO) and blood pH.

The concentration of MDA in the blood was determined by the method of Andreeva L. I. et al. (1988). Products were calculated using the molar extinction coefficient and expressed in nmol/mg. Diene conjugates and diene ketones in the blood were determined according to the method of Titeeva G.R., Korovina N.N. (1996).

The activity of SOD was determined by the method of Mkhitryan V.G. et al. (1978). The activity was calculated by the percentage of inhibition (T %) of tetrazolium blue reduction in an alkaline medium. The enzyme activity was expressed in the conv. u / min x mg protein. GPO activity was determined by the accumulation of oxidized glutathione (GSSG) as a result of the decomposition of lipid peroxides. The enzyme activity was expressed in the conv. u / min x mg Hb per minute. Erythrocyte glutathione reductase activity was determined in the reaction medium of phosphate buffer at a wavelength of

340 nm and by the decrease of NADPH * N and expressed in μM NADPH2 / min x g Hb (Vlasova S.N. et al., 1990).

Catalase activity was determined by the method of Korolyuk M.A et al. (1998), whose principle is based on the ability of H2O2 to form a resistant colored complex with molybdenum salts. The measurements were carried out at a wavelength of 410 nm. All measurements were performed on a UNICO spectrophotometer (United products and instruments, Inc., USA).

The content of hypoxia-inducible factor (HIF-1α) in blood plasma was determined by the method of enzyme-linked immunosorbent assay using Cloud-Clone corp. (USA).

Results And Discussion

The hemodynamic effect of the drug "Rheoambrasol" was studied on the model of acute blood loss, where the BP decreased to 40 mm Hg within an hour (Table 1). The volume of blood loss was 27.5 ± 2.5 mg/kg body weight. Developing hypovolemia and hypotension led to a decrease in venous return of blood to the heart, as evidenced by a reduction of central venous pressure - CVP by 76%, the minute volume of blood circulation (MVBC) by 71%, blood pressure to 40 mm Hg and maintained by increasing general peripheral vascular resistance. Total peripheral vascular resistance (TPVR) increased 1.4 times. The violation of the hemodynamic system, in turn, led to hypoxia of complex genesis, the systemic oxygen transport decreased by 4.7 times. A decrease in the blood pH level of 0.11 U was observed in parallel, the value of which was 7.31 ± 0.02 (Table 1).

Table1. Changes in the Indicators of Hemodynamics in Rabbits after Blood Loss and Infusion

Indicators	The initial state	Blood loss	Infusion of the "Rheopolyglukin"	Infusion of the "Rheoambrasol"
BP **(mm Hg)**	102,2±3,0	39,6±2,8*	89,2±2,4*^	95,8±3,7*^
CVP **(mm of water. Art.)**	12,2±2,2	5,6±2,4*	9,9±2,1^	10,1±2,3^
CBV **(ml / kg)**	55,6±2,7	32,4±2,4*	54,8±2,4^-	53,8±1,8^
MVBC **(mm Hg)**	143,5±3,0	42,1±2,7*	142,7±2,2^	146,6±6,2^
pH **(conv. U)**	7,42±0,01	7,31±0,02*	7,40±0,03^	7,41±0,03^
TPVR **(dines * s * s^{-5} / kg $x10^4$)**	5,6±1,7	7,9±1,1	4,5±1,6	4,6±1,1

SOT **(ml / min x kg)**	29,6 ±0,7	6,1±0, 8*	27,2±0,8* ^	28,0±1,0

Note: * - reliability (p <0.05) when compared with the initial state; ^ - the same (p <0.05) when compared with blood loss.

After the infusion of the drug "Rheopolyglukin" and the new infusion preparation "Rheoambrasol", the same increase in CBV was found, in all cases, which indicates the absence of the negative effect of new drugs on volemic indicators. There was an increase in CVP by 80-99% and BP by 86-96% and the MVBC after the infusion is restored to its original values. The TPVR was restored 1.3-1.4 times. After infusion of blood substitutes, the blood pH level was restored to the initial values. The value of the TPVR decreased and approached the original values of the SOT, recovering by 92-99%.

Analysis of the results showed that the infusion of a new drug led to a complete recovery of hemodynamic parameters and blood pH.

The content of hypoxia-inducible factor (HIF-1α) in the blood of experimental animals after acute blood loss increased 2.9 times (p <0.05). After the infusion of the drug "Rheopolyglukin" there was a slight tendency to a decrease in the content of HIF-1α in the blood of rats, and after the use of the drug "Rheoambrasol" the content of the factor HIF-1α decreased and approached the initial values. In group IV, there was a statistically significant decrease in the concentration of the factor HIF-1α - 1.9 times (p <0.05), which was 29.4% lower than after the introduction of the reference drug.

When studying the state of LPO in erythrocytes with blood loss, an increase in the level of diene ketones was observed 1.6 times (Table 2). The content of MDA increases significantly immediately after blood loss and after 1 hour increases 1.7 times. After blood loss, an increase in diene conjugates by 1.7 times is detected. When studying the state of AOS in erythrocytes after acute blood loss, there were no significant changes in the content of catalase, SOD, GR, and GPO, although there was a tendency to their decrease.

Table2. Changes in Lipid Peroxidation Oxidation Indicators and Antioxidant System during Acute Blood Loss and after Infusion of Blood Substitutes

Indicators	**The initial state**	**Blood loss**	**Infusion of the "Rheopolyglukin"**	**Infusion of the "Rheoambrasol"**
HIF-1α **(ng / ml)**	0,08±0 ,006	0,23±0 ,012	0,17±0,008	0,12±0,006
Catalase **(μmol / min / ml)**	6,2±0, 7	6,0±0, 7	6,1±0,7	6,5±0,7
GR **(μM NADPH2 / min x Hb)**	2,3±0, 1	2,2±0, 2	2,3±0,2	2,5±0,2
GPO **(U /**	0,3±0, 02	0,29±0 ,04	0,30±0,01	0,31±0,05

mg Hb)				
SOD **(U / mg Hb)**	2,6±0,2	2,5±0,2	2,6±0,3	2,8±0,3
MDA **(nmol / ml)**	3,2±0,06	5,4±0,1*	4,9±0,01*^	3,6±0,2^
DK **(U)**	0,17±0,09	0,27±0,02	0,26±0,2	0,19±0,1
DC **(U)**	1,2±0,1	2,04±0,2*	1,8±0,1^	1,3±0,2^
LPO / AOS **(U)**	0,42 ± 0,03	0,76 ± 0,09*	0,66 ± 0,06*^	0,49 ± 0,08^#

Note: * - reliability ($p < 0.05$) when compared with the initial state; ^ - the same ($p < 0.05$) when compared with blood loss; # - the same ($p < 0.05$) when comparing the results with the group after the infusion of the drug "Reopoliglyukin";

The results showed that blood loss is accompanied by the activation of the LPO processes, although at the same time, the state of the AOS practically does not decrease. In these studies, it was shown that, despite the blood loss, there were no significant AOS disorders, and the use of coefficient K revealed substantial changes in the LPO / AOS system. The new drug "Rheoambrasol" proved to be an effective antioxidant agent capable of restoring the LPO / AOS balance in experimental blood loss, not observed when using "Rheopolyglukin." The pronounced therapeutic effect of "Rheoambrasol" on hemodynamics and the coefficient K (LPO / AOS) in experimental blood loss is due to the presence of an energy substrate in combination with a polysaccharide. The use of the drug "Rheoambrasol" in blood loss leads to the restoration of hemodynamic parameters, an increase in oxygen transport, restoration of blood pH, and elimination of hypoxia, which leads to a decrease in the degree of intoxication, inhibition of LPO processes and stabilization of the AOS system.

Conclusions:

- The newly created drug "Rheoambrasol", containing the natural metabolite of the Krebs cycle in combination with a polysaccharide, has a good hemodynamic, anti-acidotic, antioxidant, and antihypoxant effect in acute blood loss.
- Comparative evaluation of the effectiveness of the new drug "Rheoambrasol" and "Rheopolyglukin" showed that the most effective action has a new domestic drug.

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Conflict Of Interests

All authors confirm that they have not and will not continue to make any financial claims against each other, as there is no reason for a conflict of interest. The publication of this article does not pursue commercial benefits.

Conflict of interest: not declared

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