

Comparative Evaluation of the Influence of the Solution for Infusion Therapy "Rheoambrasol" on the Functional Metabolic Parameters of Erythrocytes in Acute Intoxication Caused by a Heliotrope

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Abstract Comparatively, the effectiveness of the new solution for infusions "Rheoambrasol" on the structural and functional parameters of erythrocytes in liver damage by heliotrope was evaluated. The model of liver damage by heliotrope was reproduced in non-native (non-linear) rats. Detoxification therapy was carried out with preparations of "Rheosorbilact" or "Rheoambrasol". The activity of synthase of aminolevulinic acid (ALA synthase) and gemsynthesis in bone marrow cells, the content of uroporphyrin, coproporphyrin and protoporphyrin in erythrocytes was determined by the intensity of lipid peroxidation (LPO), the activity of antioxidant system (AOS) enzymes. A new detoxification drug improves the liver function of rats with liver damage caused by heliotrope.

Keywords Liver damage, Heliotrope, "Rheosorbilact", "Rheoambrasol", Bone marrow, Erythrocytes

1. Introduction

In recent years, despite the great successes achieved in the field of hepatology, the problem of liver damage and its pharmacotherapy remains one of the urgent problems of experimental and clinical medicine. Disclosure of many aspects of the pathogenesis of this disease allowed for the treatment of various forms of hepatitis pathogenetically justified use of membrane stabilizers, immunomodulators, antioxidants. However, a significant suppression of the activity of enzymes detoxifying the function of the liver and, as a consequence, the development of endogenous intoxication of the body, dictates the need for detoxification therapy using colloidal solutions for intravenous infusions. The most commonly used in modern clinical practice are medium molecular weight hydroxyethyl starches and solutions of succinylated or modified liquid gelatin [7, 13]. The main side effects of synthetic colloids are the effect on hemostasis and renal function, their dose-dependent effect [4]. In this regard, great importance is given to research

on the search for new non-toxic agents that would have a hemodynamic effect inherent in colloidal drugs.

Of particular importance are the work with the use of polysaccharides, which in addition to minimal toxicity have cytoprotective properties [13, 16]. Polysaccharides are able to protect cells from mechanical damage, in particular, during extracorporeal circulation, autotransfusion using "CellSaver" devices, etc..

Similar properties are due to the fact that polysaccharides are reversibly attached to erythrocyte membranes, preventing mechanical damage like plasma proteins [13].

The effectiveness of the means of infusion therapy is determined both by the specificity of the action performed by them and by the polyfunctionality of this specificity, which, taking into account the above-mentioned circumstances, determines the importance of developing preparations of complex action. Recently, much attention has been paid to bioactive polymer complexes capable of restoring metabolism in cells and affecting the vital activity of the organism as a whole. Scientists of the Scientific Research Institute of Hematology and Blood Transfusion and the Institute of Plant Substance Chemistry of the Academy of Sciences of the Republic of Uzbekistan have developed a large number of biologically active substances from plant materials capable of regulating the vital functions of cells,

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restoring the disturbed functions of organs and possessing antibiotic, antiviral, anti-tumor, antioxidant, detoxification, immunostimulating activity [13, 16].

The economic value of this class of substances is associated with their low cost and high efficiency.

As a model for investigating the effectiveness of the new domestic blood substitute in intoxication, a model of toxic liver damage was chosen, namely, an experimental model of heliotrope damage. As is known, heliotrocin is a toxicant of natural origin, containing a mixture of alkaloids and derived from the plant *Heliotropium dasycarpum* Ledeb (*Heliotrope* is pubescent), distributed in Central Asia, the Caucasus, the southeast and the European part of Russia and located in the same region states. It grows in steppes and semi-deserts, often as a field and ruderal weed. The cause of people poisoning, as a rule, is clogging of cereal seeds with heliotrope seeds, especially in the years with late spring, when the maturation of breads and heliotrope coincides (due to prolongation) [17]. Since the poisoning with heliotrocin is typical for the Central Asian and other regions, it is possible to consider intoxication with heliotrope the most natural in the experimental reproduction of liver damage, which is supported by such researchers as N. Kh. Abdullaev (1966), Fesenko, L.M. (1984), Varganova, E.I. (1984), Dombrovskaya N.V. (1993) [1, 2, 11, 14] and other authors using heliotrope intoxication as an experimental model in their studies [10].

2. Main Body

2.1. Purpose of the Study

Comparatively evaluate the effectiveness of the new solution for infusions "Rheoambrasil" on the structural and functional parameters of erythrocytes with liver damage by heliotrope.

2.2. Material and Methods of Investigation

The model of liver damage by heliotrope in 30 non-native male rats was reproduced by a single administration of heliotrocin in a dose of 40 mg / kg subcutaneously. 48 hours after administration of the toxicant (heliotrocin), the animals were divided into groups: I - (control) rats with heliotrocin hepatitis (n = 10), II - rats with heliotrope hepatitis after infusion of the drug "Rheosorbilact" (n = 10), III - rats with heliotrope hepatitis after infusion of the drug "Rheoambrasil" (n = 10). Infusion therapy was performed by introducing solutions for infusions into the tail vein to rats at a dose of 5 ml / kg body weight for 5 days. An intact group consisted of 8 rats on an ordinary laboratory diet.

To determine the status of the liver monooxygenase system, after 24 hours after the final administration of drugs in the rats, etaminal sleep was performed, according to a conventional technique that reflects the detoxifying function of the liver. 24 hours after the ethanol sleep, the animals were sacrificed under light ether anesthesia.

In the blood serum, biochemical indices were determined. The measurements were carried out on a BA88A semiautomatic biochemical analyzer (Mindray, China). Synthase activity of aminolevulinic acid (ALA-synthase) and gemseintetase in bone marrow cells was determined by the method of P.A. Kaliman et al. and expressed in nmol ALA/h per 1 mg protein and μ M protoporphyrin / min. mg of protein [6].

The content of uroporphyrin, coproporphyrin and protoporphyrin in erythrocytes was determined by the method of E. A. Shwartz in the modification of V. I. Music and expressed in nmol / l. The intensity of lipid peroxidation (LPO) in erythrocyte hemolysates was determined by the level of malonic dialdehyde (MDA) [3]. The activity of the antioxidant system (AOS) enzymes was determined: superoxide dismutase (SOD) [12], glutathione peroxidase (GPO) [5] and catalase [9]. All measurements were performed on a UNICO2800 spectrophotometer (United Products and Instruments, Inc., USA).

The digital material is processed by the method of variational statistics. Statistical processing was carried out using the programs "Excel" and "Biostat". The criterion of statistical significance was the value $p < 0.05$.

2.3. Results and Discussion

The results of the conducted studies showed that after the introduction of the heliotrope the condition of animals in group I was severe, they were sluggish, they refused to eat. The duration of etaminal anesthesia was prolonged from 72.8 ± 8.3 min in intact rats to 304.6 ± 16.8 min ($P < 0.001$) in the experimental, cytotoxic and cholestasis syndromes developed, which was confirmed by exertion of ALT and AST in the blood plasma by 351.7 ($P < 0.001$) and 456.3% ($P < 0.001$), the level of total and direct bilirubin by 193.4 ($P < 0.001$) and 255.9% ($P < 0.001$), respectively. There was a slight increase in urea levels, and the creatinine concentration increased by 16.9%.

One of the main causes of cell death in the case of the action of the hepatotoxic agent is the depletion of the system of energy production and detoxification of xenobiotics. In this regard, a very promising direction in medicine is the creation of tools that restore the metabolism of hepatocytes in various pathological conditions [8, 15]. Carrying out detoxification therapy with "Rheosorbilact" or "Rheoambrasil" preparations, the animals' condition improved, tearing, salivation ceased, animals became active. The activity of ALT significantly decreased in 3 and 3.97 times, AST - in 2.9 and 3.5 times, total bilirubin - in 2.14 and 2.52 times, direct bilirubin - in 2.88 and 3.56 times, respectively. The results of these studies correlated with the parameters of etaminal sleep, which, after the application of solutions for infusions, was shortened by 2 and 3.1 times, respectively, to the groups receiving "Rheosorbilact" and "Rheoambrasil". As can be seen from the data presented, the use of the new domestic preparation "Rheoambrasil" accelerates the excretion of toxins from the body, reducing

the cytolytic manifestations of heliotrines, and in its detoxification activity it is not inferior to the widely used "Rheosorbilact".

Along with this, we observed the intensification of LPO, which was manifested by an increase in the level of MDA in the erythrocyte hemolysates by 2.4 times ($P < 0.001$) (Table 1).

Table 1. Change in the indices of lipid peroxidation and antioxidant system during heliotrope intoxication in rats and after detoxification therapy ($M \pm m$)

Investigated indicators	Intact animals	Intoxication caused by heliotrope
Catalase, nm / mg Hb • min	42,1 $\pm$ 2,6	26,2 $\pm$ 2,8
GPO, U / min • mg Hb	0,31 $\pm$ 0,03	0,11 $\pm$ 0,04*
SOD, U / mg Hb	2,1 $\pm$ 0,2	1,1 $\pm$ 0,3*
MDA, nM / mg Hb	0,5 $\pm$ 0,04	1,2 $\pm$ 0,08*
Investigated indicators	Intoxication caused by heliotrope + infusion of solutions for infusions	
	"Rheosorbilact"	"Rheoambrosol"
Catalase, nm / mg Hb • min	32,5 $\pm$ 3,1*^	38,2 $\pm$ 4,2
GPO, U / min • mg Hb	0,20 $\pm$ 0,05^	0,28 $\pm$ 0,04^
SOD, U / mg Hb	1,7 $\pm$ 0,3	1,9 $\pm$ 0,2^
MDA, nM / mg Hb	0,6 $\pm$ 0,04^	0,5 $\pm$ 0,05*^#

Note: * - reliability ($p < 0,05$) when compared with the initial state; ^ - the same ($p < 0,05$) when compared with the indices of the group with intoxication caused by heliotrope; # - the same ($p < 0,05$) when comparing the results with the group after infusion of the solutions for infusions "Rheosorbilact".

This process proceeded against the background of a decrease in the activity of SOD, GPO and catalase in erythrocytes of blood in 1.9 ($P < 0.001$); 2.8 ($P < 0.001$) and 1.6 ($P < 0.001$) times, respectively. Disintoxication pharmacotherapy with "Rheosorbilact" and "Rheoambrosol" drugs reduced the severity of lipid peroxidation, which was manifested by a significant decrease in the level of MDA in 2 and 2.4 times, restoration of activity of enzymes AOP and balance in the LPO / AOS system.

Apparently, the stabilization of biological membranes under the influence of detoxification therapy is associated with the leaching of toxic metabolites of endogenous and exogenous origin, which contributes to the maintenance of homeostasis. This is confirmed by the increased activity of the pharmaco-metabolizing function of hepatocytes, since the duration of etaminal anesthesia was substantially shorter.

A more pronounced normalization of the homeostatic parameters of experimental animals by the action of the drug "Rheoambrosol", in our opinion, is due to the presence of succinic acid in its composition, which contributes to the improvement of energy supply of cells. On the other hand, biologically active compounds of plant origin, included in its composition, as well as plant heteropolysaccharides possess cytoprotective properties [13, 16]. They, like blood plasma proteins, reversibly bind to erythrocyte membranes and protect them from mechanical damage [7].

In liver lesions, the development of anemia is noted, due to the systemic action of viruses on biological membranes, and also, as a result of increased hemoglobin breakdown. In this

connection, it was of interest to evaluate the effect of detoxification preparations on the parameters of heme synthesis and decomposition in bone marrow cells, as well as the products of its metabolism in erythrocytes (Table 2, 3). The performed studies showed compensatory activation of the key regulatory enzyme for heme – ALA-synthase synthesis in 1,3 times in response to a decrease in hemoglobin level due to low activity of gemsinase in rats with heliotrope hepatitis. Thus, it was possible to observe a significant decrease in the activity of gemsynthase in 1.87 times, relative to the values of this index in intact animals. At the same time, in the erythrocytes, all the intermediate products of heme synthesis were reduced: protoporphyrin, uroporphyrin, coproporphyrin and all porphyrins – in 1.22; 1.49; and 1.44 times, respectively (Table 2).

Table 2. Change in heme synthesis in erythrocytes in hepatitis caused by heliotrope intoxication and after infusion of solutions for infusions ($M \pm m$)

Erythrocytes		
Investigated indicators	Intact animals	Intoxication caused by heliotrope
Protoporphyrin, nmol / ml	249,5 $\pm$ 10,6	204,7 $\pm$ 19,3*
Uroporphyrin, nmol / ml	9,1 $\pm$ 0,6	6,1 $\pm$ 0,5*
Coproporphyrin, nmol / ml	90,9 $\pm$ 6,0	63,1 $\pm$ 5,9*
Total porphyrins, nmol / ml	349,5 $\pm$ 28,7	273,9 $\pm$ 25,7*
Indirect bilirubin, μ mol / l	12,1 $\pm$ 0,8	34,0 $\pm$ 1,8*
Investigated indicators	Intoxication caused by Heliotrope + infusion of solutions for infusions	
	"Rheosorbilact"	"Rheoambrosol"
Protoporphyrin, nmol / ml	221,6 $\pm$ 20,8	258,6 $\pm$ 15,6^
Uroporphyrin, nmol / ml	7,2 $\pm$ 0,6*	9,0 $\pm$ 0,6^, #
Coproporphyrin, nmol / ml	73,8 $\pm$ 6,8*	90,4 $\pm$ 6,8^, #
Total porphyrins, nmol / ml	322,6 $\pm$ 28,2	358,0 $\pm$ 23,0^
Indirect bilirubin, μ mol / l	17,3 $\pm$ 0,9*, ^	14,9 $\pm$ 0,7^

Note: * - reliability ($p < 0,05$) when compared with the initial state; ^ - the same ($p < 0,05$) when compared with the indices of the group with intoxication caused by heliotrope; # - the same ($p < 0,05$) when comparing the results with the group after infusion of the solution for infusions "Rheosorbilact".

Table 3. Changes in heme synthesis in the bone marrow in heliotrope hepatitis and after infusion therapy with solutions for infusions ($M \pm m$)

Bone marrow		
Investigated indicators	Intact animals	Intoxication caused by heliotrope
ALA-synthase, nmol / h	0,328 $\pm$ 0,012	0,426 $\pm$ 0,011*
Gemsynthetase, μ mol / min • mg	0,241 $\pm$ 0,011	0,129 $\pm$ 0,003*
Investigated indicators	Intoxication caused by Heliotrope + infusion of solutions for infusions	
	"Rheosorbilact"	"Rheoambrosol"
ALA-synthase, nmol / h	0,360 $\pm$ 0,022^	0,325 $\pm$ 0,022^
Gemsynthetase, μ mol / min • mg	0,131 $\pm$ 0,003	0,172 $\pm$ 0,003*^

Note: * - reliability ($p < 0,05$) when compared with the initial state; ^ - the same ($p < 0,05$) when compared with the indices of the group with intoxication caused by heliotrope; # - the same ($p < 0,05$) when comparing the results with the group after infusion of the solution for infusions "Rheosorbilact".

The rate of heme formation, on the one hand, depends on the activity of the key enzyme for heme-aminolevulinatase synthase synthesis, and at another rate of its destruction by hemoxygenase, which can be manifested by high values of indirect bilirubin. Indeed, we observed a sharp increase in the level of indirect bilirubin by 2.81 times. The obtained results testify both to the slowing down of the synthesis processes and to the intensification of the process of decomposition of hemoglobin in the organism of experimental animals. The data obtained by us coincide with the literature data, which shows the development of the hematological syndrome ("syndrome of hypersplenism"), manifested by the development of anemia, thrombocytopenia and leukopenia.

The desintoxication therapy of heliotrope hepatitis with "Rheosorbilact" and "Rheoambrasol" led to a decrease in the activity of ALA-synthase in 1.18 and 1.31 times, an increase in the activity of hem-synthase only in the group receiving "Rheoambrasol" 1.33 times. The increase in protoporphyrins, uroporphyrins, coproporphyrins and the amount of porphyrins in erythrocytes of blood, as well as the content of indirect bilirubin in blood plasma. The obtained data testify to an increase in the functional activity of bone marrow cells and suppression of erythrocyte decay during detoxification therapy. This was more pronounced when using the drug "Rheoambrasol".

Thus, we can say that the use of a new solution for infusions "Rheoambrasol" leads to the stabilization of cell membranes, an increase in the activity of antioxidant defense enzymes, which ultimately leads to the restoration of the synthetic functions of bone marrow cells, to a decrease in the decay of erythrocytes. In our opinion, this is due to the presence in its composition of succinic acid, the natural metabolite of the Krebs cycle [8]. Studies of scientists have proved the exceptional importance of natural metabolites as the energy most valuable substrate, of which succinic acid is most effective in hypoxic conditions [15]. Adaptation of the body to hypoxia, according to the authors, is associated with a transition to the accumulation and use of succinic acid, and in conditions of a decreased partial pressure of oxygen in the body, its oxidation is much more effective [15]. In experimental and clinical studies, the high efficiency of sodium succinate in hypoxia [8].

3. Conclusions

Based on the data obtained, the following conclusions can be drawn:

1. A new detoxification drug improves the liver function of rats with heliotrope liver damage, especially its pharmaco-metabolizing function.
2. "Rheoambrasol" has an antioxidant effect, increases the low activity of SOD, GKO and catalase enzymes in erythrocytes, and protects them from hyperlipoperoxidation.

3. "Rheoambrasol" stabilizes the functional metabolic parameters of bone marrow cells, which is manifested by the restoration of hemoglobin-synthesizing activity, an increase in the content of porphyrins.

REFERENCES

- [1] Abdukadyrovna S. R., Usmanovna K. M. 2015, Mechanism of the development of apoptosis in the mucosa of the gastrointestinal tract in the mixed pathology, European science review. 11-12.
- [2] Alimova L. A., Begmanov S. A., Nigmatov N. N.; Abidova N. A. 2015, Some aspects of infectious-allergic and toxic hepatitis and cirrhosis // Novosibirsk State Pedagogical University Bulletin, 1, p. 80-87. 8p.
- [3] Andreeva L. I., Kozhemyakin L. A., Kishkun A. A. 1988, Modification of the method for the determination of lipid peroxides in the test with thiobarbituric acid., Laboratory work, 1, 41-43.
- [4] Bulanov A. Yu., Gorodetsky V. M., Shulutko E. M. Colloidal void-replacing solutions and hemostasis. - Moscow., 2003.
- [5] Vlasova S. N., Shabunina E. I., Pereslegina I. A., 1990, Activity of glutathione-dependent erythrocyte enzymes in chronic liver diseases in children., Laboratory work., 8, 19-22.
- [6] Kaliman P. A., Belovatskaya I. V. 1986, Influence of cobalt chloride on the activity of key enzymes of heme metabolism in rat liver., Biochemistry, 8(51). 1302-1307.
- [7] Karimov Kh. Ya., Shevchenko L. I., Rakhmanberdieva R. K., Zaidova Sh. B., Alimov T. R. 2012, Application of polysaccharide complexes from plant raw materials in the composition of blood substitutes., Medical Journal of Uzbekistan, - Tashkent, 6, 88-91.
- [8] Kondrasheva M. N., Kaminsky Yu. T. Amber acid in medicine, food industry, agriculture., Pushchino: ONTRAMN, 1996. – 300 p.
- [9] Koralyuk M. A., Ivanova L. I., Mayorova I. G. 1988, Determination of catalase activity., Laboratory work. 1, 16-19.
- [10] Kulmanova M. Y., etc. 2013, Oxidant-antioxidant and microsomal systems in case of salmonellosis infection against heliotricin hepatitis // News of problems in biology and medicine, 1(4), 157-160.
- [11] Mahmadaliev Kh. Zh. et al. 2010, The hepatoprotective action of stimulosin in experimental chronic hepatitis // Siberian Medical Journal (Irkutsk), 99 (8).
- [12] Mkhitarian V. G., Badalyan G. Ye. 1978, Influence of peroxidized and non-peroxidized unsaturated fatty acids on the activity of superoxide dismutase, Zh. expert. and wedge. med., 6, 7-11.
- [13] Rakhmanberdieva R. K., Rakhimov D. A., Nigmatullaev A. 2010., Patent "The method of obtaining a polysaccharide to create on its basis a density gradient medium" JAP # 04111. Official bulletin [Rasmij abhorotnoma].; 3.

- [14] Sabirov R. A., Kulmanova M. Y. 2014, The state of cytokine status in experimental heliotrogenic hepatitis // Clinical medicine of Kazakhstan. 4 (34).
- [15] Stelmakh V. V., Radchenko V. G., Kozlov V. K. 2011. Metabolic correctors on the basis of succinic acid as a means of pathogenetic therapy for chronic viral hepatitis. Scientific and practical journal., 2, p. 67.
- [16] Shevchenko L. I., Rakhmanberdieva R. K., Karimov Kh. Ya., Alimov T. R. 2014. Expediency of the use of polysaccharides in the composition of blood substitutes for hemodynamic action in cases of hemorrhagic shock., Journal of Theoretical and Clinical Medicine., 1., 97-101.
- [17] Zobnin Yu. V. 2017, Toxic liver damage in children // Siberian Medical Journal (Irkutsk), 151 (4), p. 37-53, 50p.