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Antioxidant Protection of Donor Packed Red Blood Cells using Mexidol

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Abstract

The current research shows the possibility of long-term conservation of the erythrocytes an-tioxidant activity in the process of donor packed red blood cells while introducing mexidol to the hemoconservant glugicir composition.

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Introduction

Storage and supply of blood components for possible emergency disaster situation is one of the main tasks of emergency disaster medicine.

While donor blood and its components storage, the viability, morphological and functional values of erythrocytes are gradually decreasing. Alterations in structural and functional organization of cells membranes during donor blood components storage and failure in functioning of membrane connected enzymes and excessive synthesis of free radicals inevitably leads to accelerated blood cells "ageing" [1, 2]. Hence, correction of pathophysiological changes in blood transfusion environment at the stage of blood storage is our priority task [3].

We consider creation of new hemopreservatives and further development of the existing ones as the most important trends in transfusiology which allows raising functional value of erythrocytes, medical efficiency of blood transfusions, and lasting blood components storage time [4, 5].

The overall aim of this research is to investigate the capacity of long-term preservation of the erythrocyte native state in donor packed red blood cells (RBC) by inactivating the processes of lipid peroxygenation (LP) and correction of antioxidant activity (AAC) in erythrocytes with standard RBC storage (at t + 4 ^{0}C), by adding 2-ethyl-6-methyl-3-hydroxypyridine succinate antioxidant to composition of the blood conversant "Glugicir".

Materials and Methods

We prepared several samples of packed RBC on glugicir preservative which contained 0.25 mg/ml, 0.5 mg/ml and 1.0 mg/ml 2-ethyl-6-methyl-3-hydroxypyridine succinate antioxidant, respectively. As a control sample we used packed RBC prepared on glugicir with no supplements. We examined the biochemical values of erythrocites in the packed RBC samples immediately after their preparation and in during its storaging at the t = + 4 ^{0}C for 6 hours, 3 days, 7 days, 14 days, 21 days and 30 days.

The AAC intensity has been estimated by concentration of malondialdehyde (MDA), which was defined by the reaction with 2-thiobarbituric acid, according to the L.I. Andreeva's method [6]. The intensity of the Free Radical Oxidation (FRO) and General Antioxidant Activity (GAA) were assessed by the chemiluminometer Emilite-1105 (Latvia). The level of catalase was defined according to the M.A. Koroluk's and Co method) [7]. The method based on the ability of hydrogen peroxide to form a stable colored complex with molybdate salts. We applied a modification of this technique, which imply selection the optimum amounts of ammonium molybdate and hydrogen peroxide by titration on the amount of hydrogen peroxide in the solution). 10 ml of 4% solution of ammonium molybdate (0.4 mg per sample volume in a cuvette) was used. The optimal amount of hydrogen peroxide was selected by titration method, the molar concentration of hydrogen peroxide in the solution was calculated: 30 µl of 4% solution of hydrogen peroxide with total content of H_2O_2 of 1.2 mg was used.

Results and Discussion

The intensification of FRO was observed since the first hours of the packed RBC storage. The greatest increase of FRO by 168 % ($p<0.0001$) was detected in the sample of the packed RBC stored with 0.25 mg/ml 2-ethyl-6-methyl-3-hydroxypyridine succinate while in samples with higher concentrations of 2-ethyl-6-methyl-3-hydroxypyridine succinate (0.5 mg/ml and 1.0 mg/ml) FRO observed to be decreasing by 16 % and 4 %, respectively (Table. 1).

By the end of the first week of storage the FRO activity in all test samples was significantly lower than in control samples. Within the group with 1.0 mg/ml concentration of 2-ethyl-6-methyl-3-hydroxypyridine succinate the FRO activation was observed to be increased by 37 % ($p<0.001$) and 48 % ($p<0.001$) comparatively to the samples with concentration of 2-ethyl-6-methyl-3-hydroxypyridine succinate in hemostabilizer 0.25 mg/ml and 0.5 mg/ml, respectively.

The antioxidant activity in control samples has decreased by 57 % ($p<0.001$) compared with initial values by 30 day of storage. GAA into the packed RBCs with added 2-ethyl-6-methyl-3-hydroxypyridine succinate at a dose of 0.25 mg/ml remained on the initial level by the third day. By the end of the first week, it gradually started decreasing and reached the seven-day level of control samples by the 30th day. The

Table 1. FRO activity, relative unit

Hemotransfusion medium	Outcome	6 hours	3 days	7 days	14 days	21 days	30 days
Packed RBC (glugicir)	0,664±0,11	0,908±0,17	2,44±0,06 p_1<0,0001	3,14±0,07 p_1<0,0001	3,35±0,07 p_1<0,0001	3,59±0,06 p_1<0,0001	4,11±0,08 p_1<0,0001
Packed RBC (glugicir +2-ethyl-6-methyl-3- hydroxypyridine succinate 0.25 mg/ml)		1,871±0,01 p_1<0,0001 p_2<0,0001	1,69±0,04 p_1<0,0001 p_2<0,001	1,51±0,06 p_1<0,0001 p_2<0,0001	1,41±0,07 p_2<0,0001	1,73±0,03 p_1<0,0001 p_2<0,0001	2,04±0,06 p_1<0,0001 p_2<0,0001
Packed RBC (glugicir +2-ethyl-6-methyl-3- hydroxypyridine succinate 0.5 mg/ml)		1,016±0,02 p_3<0,0001	1,18±0,05 p_2<0,0001 p_3<0,0001	1,52±0,04 p_1<0,0001 p_2<0,0001	2,46±0,06 p_1<0,0001 p_2<0,000 1p_3<0,0001	3,19±0,05 p_1<0,0001 p_2<0,0001 p_3<0,0001	2,45±0,04 p_1<0,0001 p_2<0,0001 p_3<0,0001
Packed RBC (glugicir +2-ethyl-6-methyl-3- hydroxypyridine succinate 1.0 mg/l)		0,924±0,05 p_3<0,0001 p_4<0,05	2,09±0,06 p_1<0,0001 p_2<0,05 p_4<0,0001	2,28±0,08 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,0001	2,23±0,04 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,0001	2,48±0,03 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,0001	2,66±0,02 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,0001

Notice: P_1 - significance of differences with the initial data; P_2- significance of differences with the control serial data; P_3- significance of differences with the serial data, containing 2-ethyl-6-methyl-3-hydroxypyridine succinate in concentration of 0.25 mg/ml; P_4- significance of data with serial data, containing 2-ethyl-6-methyl-3-hydroxypyridine succinate in concentration of 0.5 mg/ml.

Table 2. Antioxidant activity, relative unit.

Hemotransfusion medium	outcome	6 hours	3 days	7 days	14 days	21 days	30 days
Packed RBC (glugicir)	2,07±0,048	2,03±0,053	1,78±0,044 p_1<0,0001	1,74±0,037 p_1<0,0001	1,52±0,081 p_1<0,0001	1,26±0,065 p_1<0,0001	0,89±0,024 p_1<0,0001
Packed RBC (glugicir +2-ethyl -6-methyl-3- hydroxypyridine succinate 0.25 mg/ml)		2,08±0,031	2,34±0,030 p_1<0,0001 p_2<0,0001	2,08±0,018 p_2<0,0001	1,90±0,022 p_1<0,0001 p_2<0,0001	1,67±0,022 p_1<0,0001 p_2<0,0001	1,44±0,010 p_1<0,0001 p_2<0,0001
Packed RBC (glugicir +2-ethyl -6-methyl-3- hydroxypyridine succinate 0.5 mg/ml)		2,35±0,039 p_1<0,0001 p_2<0,0001 p_3<0,0001	2,42±0,058 p_1<0,0001 p_2<0,0001	2,15±0,017 p_1<0,0001 p_2<0,0001 p_3<0,05	2,69±0,011 p_1<0,0001 p_2<0,0001 p_3<0,0001	2,81±0,015 p_1<0,0001 p_2<0,0001 p_3<0,0001	2,36±0,023 p_1<0,0001 p_2<0,0001 p_3<0,0001
Packed RBC (glugicir +2-ethyl -6-methyl-3- hydroxypyridine succinate 1.0 mg/l)		2,11±0,013 p_4<0,0001	2,28±0,025 p_1<0,0001 p_2<0,0001 p_4<0,05	1,97±0,018 p_2<0,0001 p_3<0,0001 p_4<0,0001	2,54±0,015 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,0001	2,79±0,017 p_1<0,0001 p_2<0,0001 p_3<0,0001	2,45±0,009 p_1<0,0001 p_2<0,0001 p_3<0,0001 p_4<0,001

Note: p_1- significance of differences with the initial data, p_2- significance of differences with the control serial data, p_3- significance of differences with the serial data, containing 2-ethyl-6-methyl-3-hydroxypyridine succinate in concentration of 0.25 mg/ml, p_4- significance of data with serial data, containing 2-ethyl-6-methyl-3 -hydroxypyridine succinate in concentration of 0.5 mg/ml.

Table 3. Catalase activity (mcaT/l).

Hemotransfusion medium	outcome	6 hours	3 days	7 days	14 days	21 days	30 days
Packed RBC (glugicir)	12,7±1,53	14,42±1,48	15,5±1,38 p_1<0,05	25,6±1,21 p_1<0,0001	31,8±3,15 p_1<0,0001	24,50±1,59 p_1<0,0001	26,4±0,22 p_1<0,0001
Packed RBC (glugicir +2-ethyl-6-methyl-3-hydroxypyridine succinate 0.25 mg/ml)		21,9±0,32 p_1<0,0001 p_2<0,0001	24,0±0,28 p_1<0,0001 p_2<0,05	29,2±0,51 p_1<0,0001	30,1±0,60 p_1<0,0001	28,894±0,62 p_1<0,0001	28,5±0,54 p_1<0,0001
Packed RBC (glugicir +2-ethyl-6-methyl-3-hydroxypyridine succinate 0.5 mg/ml)		19,5±0,35 p_1<0,0001 p_2<0,0001	22,5±0,18 p_1<0,0001 p_2<0,0001 p_3<0,0001	28,4±0,28 p_1<0,0001 p_3<0,005	29,4±0,41 p_3<0,0001	28,871±0,59 p_1<0,0001 p_2<0,05 p_3<0,0001	27,5±0,65 p_1<0,0001 p_3<0,01
Packed RBC (glugicir +2-ethyl-6-methyl-3-hydroxypyridine succinate 1.0 mg/l)		21,5±0,27 p_1<0,0001 p_2<0,0001	23,0±0,34 p_1<0,0001 p_2<0,001 p_3<0,0001 p_4<0,0001	29,15±0,32 p_1<0,0001 p_2<0,05 p_4<0,0001	30,8±0,32 p_1<0,0001 p_2<0,05 p_3<0,0001 p_4<0,0001	28,229±1,16 p_1<0,0001 p_4<0,01	29,7±1,03 p_1<0,0001 p_4<0,005

Note: p_1- significance of differences with initial values, p_2- significance of differences with control series data, p_3- significance of differences with the series with 2-ethyl-6-methyl-3-hydroxypyridine succinate in concentration of 0.25 mg/ml, p_4- significance of differences with the series with 2-ethyl-6-methyl-3-hydroxypyridine succinate in concentration of 0.5 mg/ml.

packed RBCs samples that containined 2-ethyl-6-methyl-3-hydroxypyridine succinate 0.5 mg/ml during the whole storable life, had the GAA level higher than the control sample, and this level had increased by 166 % ($p<0.0001$) higher than in the samples with the 2-ethyl-6-methyl-3-hydroxypyridine succinate 0.25 mg/ml concentration by the 30th day of storage. 2-ethyl-6-methyl-3-hydroxypyridine succinate at a dosage of 1.0 mg/ml in the packed RBCs was able to activate GAA during the whole period of storage, while at a dosage of 0.25 mg/ml the efficacy of the antioxidant effect by the 14-th day of storage decreased (Table 2).

The catalase activity in the control samples of the packed red blood cells kept growing for the two weeks of storage and reached 151% ($p<0.001$), reducing insignificantly by the end of the storage term comparing to the initial values. In the samples of the packed RBCs with 2-ethyl-6-methyl-3-hydroxypyridine succinate we observed the significant growth of catalase activity, increased by the 14th day up to 138 % ($p<0.0001$) and up to 161 % ($p<0.0001$) in the packed RBCs with 2-ethyl-6-methyl-3-hydroxypyridine succinate 0.25 mg/ml and 0.5 mg/ml concentration in hemostabillizer, by the 30th day with control samples (Table 3).

It was observed that the packed RBCs, when stored, underwent the intensive decrease of the antioxidant activity protection, activating lipid peroxidation. Applying the exogenic antioxidant 2-ethyl-6-methyl-3-hydroxypyridine succinate allows to decrease the lipid peroxidation activity and the "load" on antioxidant erythrocyte systems. Introduction of 2-ethyl-6-methyl-3-hydroxypyridine succinate into

hemoconservant causes the evident membrane-protective action and allows adapting blood corpuscles to their lives in the conditions of hypoxia. The minimal level of lipid peroxidation activation has been defined in the erytheromass, while stored with 2-ethyl-6-methyl-3-hydroxypyridine succinate in 0.25 mg/ml glugicir concentration; however, antioxidant activity was lower than in other experimental samples.

Possibly, the activation of lipid peroxidation in the groups with the 2-ethyl-6-methyl-3-hydroxypyridine succinate 0.5 and 1.0 mg/ml glugicir concentration is linked to the inhibition of endogenic antioxidants of RBCs while introducing large dosage of exogenic antioxidants based on principles of biofeedback.

Conclusion

Based on the obtained results, we advise to prepare packed RBCs on glugicir, containing 2-ethyl-6-methyl-3-hydroxypyridine succinate in its minimal concentration — 0.25 mg/ml glugicir.

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