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Mossbauer study of different factors influence on donated blood quality

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Abstract Packed red blood cells (RBC) are main blood components used in transfusiology. During storage, preserved blood cells undergo progressive structural and functional changes that may reduce red-cell function and viability after transfusion. As the hemoglobin is the main and active component of erythrocytes, we consider that oxidized hemoglobin derivatives formation cause those changes. With the aim of developing the new criteria for donated blood suitability for transfusion, we studied storage-related changes in red blood cells hemoglobin by means of analytical fitting of Mössbauer spectra. We analyzed Mössbauer spectra of set RBC's samples from volunteer donors every few days during their storage. Using those data, we built kinetic curves of various hemoglobin derivatives changes during RBC storage for different hemoglobin forms concentration in RBC samples. We found that kinetic curves are specific for each donor. They also depend on the donor's age and health condition at the time of donation. Based on our spectroscopy results we suggested an objective criteria for duration of storage of regular donors RBC.

Keywords Mössbauer spectroscopy · Blood donation · Red blood cells · Hemoglobin derivatives

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1 Introduction

Red blood cells (RBCs) are the most important blood components used in transfusion medicine [1]. They are produced by separating erythrocytes from whole blood and mixing with anticoagulant-preservative solution.

Red blood cell transfusions are performed to raise the hematocrit level in patients with anemia or to replace losses after acute bleeding episodes. Usually in vivo in bloodstream erythrocytes stay alive throughout 120 days but packed red blood cells keep viability only from 35 to 41 days (depending on preserving agent). The point is that erythrocytes are removed from natural medium. The blood preserving agent induces metabolic and energy damage in RBC only partially.

Reductive-oxidative erythrocyte state is the principal cell metabolism regulator. This state variation induced by external or internal actions changes cell function. It is regulated by different antioxidant defense mechanism provided by B- and E- vitamin, glutathione, carotenoid metabolites, lithic acid, scavenging enzymes. During the packed red blood cells storage the lack of native defense mechanism brings about iron ions oxidation in hemoglobin molecule.

As a result of this process we have formation of methemoglobin which is not able to bind oxygen. Subsequent hemoglobin oxidative damage results in hemichrome and haemin formation which are the irreversible oxidative hemoglobin derivatives [2]. It really leads to trivalent iron ions release from the denatured hemoglobin and to its linking with an erythrocyte membrane, and further to membrane damage.

Transfusion of packed red blood cells with the oxidized forms high concentration as showed by biomedical statistical researches becomes unsafe [3, 4]. Therefore it was of interest to determine the time of crucial undesirable transformations in packed RBC and how this time correlates with standard duration of storage and varied donor's individual features (age and health at the moment of donation) .

Hemoglobin is the main filling protein in red blood cells. Thus the main red cell functions directly depend on hemoglobin state changes. To determine the hemoglobin transformations during RBC storage we used the possibilities of their Mössbauer spectra quantitative analysis. Mössbauer spectroscopy - an extremely sensitive and selective method for detecting the valence and spin state changes of iron in active center of hemoglobin molecule. Iron-containing biological molecules and tissues have been the objects of Mössbauer researches since Mössbauer Effect discovery [5–7]. The high resolution of the method (10^{-13}) allows to distinguish all hemoglobin derivatives resulting in ageing and degradation of red blood cells and to obtain quantitative correlation with high accuracy. This is due to the fact that the resolution of this method is higher by several orders of magnitude than optical methods which are currently used in biochemical studies [4]. It is important to note that the method is noninvasive and requires no special chemical treatment of red blood cells. By now Mössbauer spectra of almost all hemoglobin forms and derivates have been obtained and their parameters are well known [8–16].

In this paper we study the quantitative changes of packed red blood cells state at different stages of storage via variable factors (like donor volunteer age, state of health at the moment of donation) by means of Mössbauer spectroscopy.

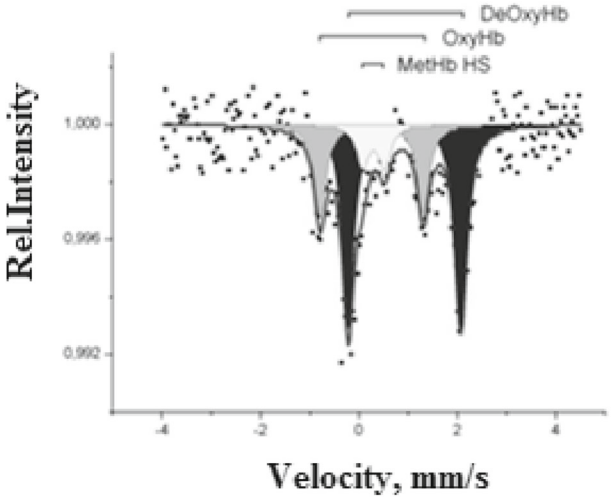

Fig. 1 Mössbauer spectrum of fresh donor's RBC

2 Experimental

2.1 Samples

We investigated a set of standard red blood cells samples kept in standard containers and stabilized by preserving agent CPDA-1 (their standard time of storage 35 days at 4 °C). Besides that we analyzed the samples of fresh red blood cell suspension with added of anticoagulant, the once that are usually used in authotransfusion in clinics. These samples were received from 15 volonteer donors of different age and state of health (from 20 to 60 years old). All the samples under investigation were stored in hermetically sealed vial in the refrigerator at 4 °C during the entire time of the experiments.

Mössbauer spectra were recorded on a Mössbauer spectrometer MS 1104 Em ("Kordon") using radioactive Co^{57} source with activity of 50 mCi. Mössbauer spectra of the RBC's survey from each container was held every few days for the duration of storage of red cells (35 days). Every time donated RBC samples in volume of 1 ml were extracted from a container, placed in a sterile, airtight plastic sample cell for Mössbauer measurements. The sample cell was immediately fast cooled down and then placed in a low-temperature cryostat for Mossbauer measurements at 80K.

Analytical treatment of received Mössbauer spectra was done by fitting with sum of different subspectra with Lorentzian line shape corresponding to various hemoglobin derivatives. Subspectra areas are related to hemoglobin derivatives quantities. The kinetic curves were plotted to reflect changes in the content of active and trivalent forms of oxidized hemoglobin in donated blood of particular donor during its storage for 35 days. Analysis of these forms content kinetic dependencies over time made it possible to make conclusions of erythrocytes functional state changes in RBCs of a regular donor.

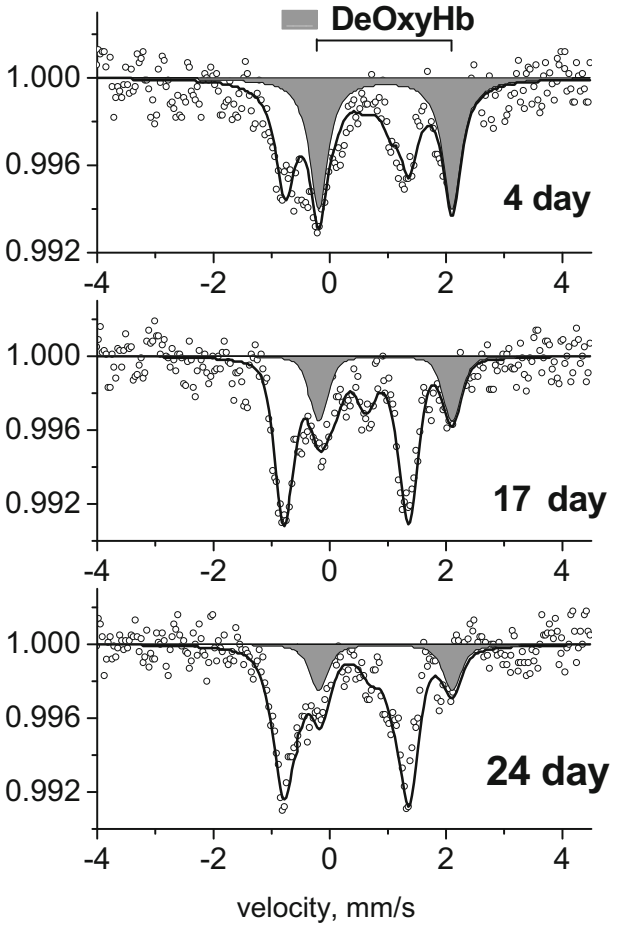

Fig. 2 Mössbauer spectra of standard RBC obtained at different stages of its storage

2.2 Experimental results

To evaluate the efficiency and sensitivity of our approach we compared oxidative process kinetics for two sample types: the red blood cell suspension and standard packed red blood cells. Red blood cell suspension is produced from whole blood by centrifugation, removing buffy coat and adding not preserving agent but anticoagulant. Clinically, red blood cell suspension is being stored no more than 3 days and usually is used for autotransfusion. Mössbauer spectrum of fresh donor's RBC is shown in Fig. 1. It is represented by two intensive doublets corresponding to oxyhemoglobin (oxyHb) and deoxyhemoglobin and a small doublet of ferric methemoglobin (metHb). Usually standard RBC samples contain no more 60% of deOxyHb because venous blood cells partially oxydize during deriving from the whole blood.

Packed red blood cells stabilized by hemoconvant can be stored for 35 days. The hemoconvant application slows down oxidizing processes significantly. From Mössbauer spectra (Fig. 2) of standard RBC obtained at different stages of its storage we can deduce the main

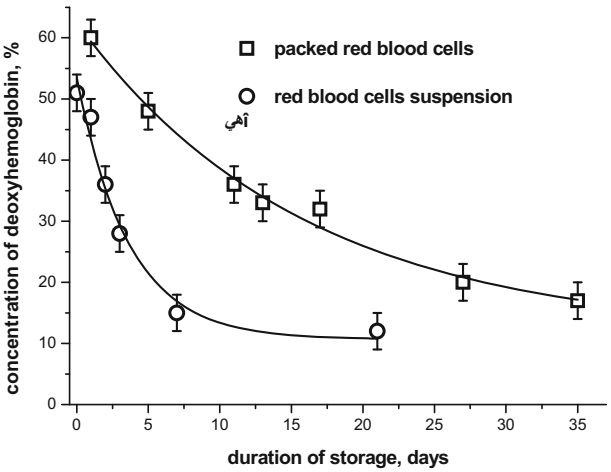

Fig. 3 Deoxyhemoglobin concenration kinetics change during storage for two types of RBC samples

and most resolved component (DeOxyHb) changes. This component intensity changes may be used to construct kinetic curves for donated material degradation.

To test the use of this component as a marker of RBC degradation process we compare two kinetic curves from two types of RBC samples: fresh and standard packed. The kinetic curves of the main component - deoxyHb- concentration changes during storage for those two RBC types are plotted in Fig. 3. Both curves have an exponential character.

The kinetic curve corresponding to the sample of red blood cell suspension has a large rate of deoxyHb decline. The ratio of deoxyhemoglobin in it reduces to 35% on the third day of blood storage and on the seventh day it is only 12%. A similar curve for the second sample undergoes a more gradual decline: the concentration of the active form is reduced twofold in the middle of standard storage duration and at the end of the storage it is 17%.

These kinetic curves indicate a high sensitivity of the developed technique to the deoxyhemoglobin concentration in the samples under study. A common to all donors trend was detected during research. It consists in significant decrease of deoxyHb concentration in donated blood over time. Mössbauer spectra of red blood cells suspension of the same donor in varied states of health are presented in Fig. 4a, b. It is clearly seen that concentration of deoxyHb in blood in case of semi-diseased condition is very low.

The kinetic curves characteristic for different donors also markedly differ. All these curves are the exponential dependence with a different exponential index. DeoxHb reduction kinetics for the donors (marked 5,6) has near-linear dependence (Fig. 5a). The kinetic curve for the donor (marked 4) significantly differs from 1-3. On the first day of storage concentration of deoxyHb is only 35%. The figures show that deoxyHb concentration significantly decreases in red blood cells at about the middle of the standard storage period (15 - 20 days). Its concentration is about 50% of the original value. It is obviously that kinetics of these processes vary for RBCs from different donors.

The decrease in hemoglobin content in the samples is due to the formation and increase in the proportion of hemoglobin trivalent oxidized forms (methemoglobin, hemichrom, hemin). It can be seen from Fig. 6 that in the first week of storage, their content is insignificant (0-10%), however, after 2 weeks, their concentration in the sample is increased from 15 to 25% and then at the end of the shelf life their share is 25-30 %.

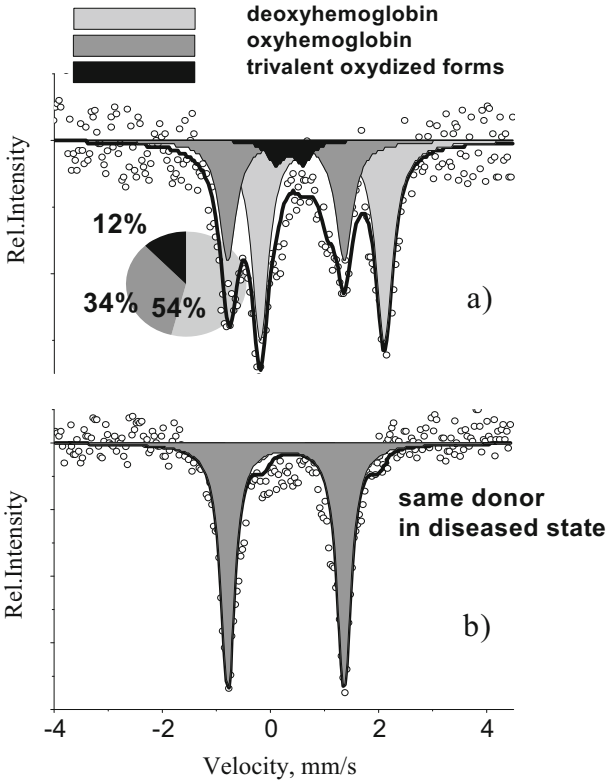

Fig. 4 Mössbauer spectra of RBC suspension from one donor in various health conditions

Kinetic curves of DeOxyHb degradation in fresh RBC of different age donor group (Fig. 7) demonstrate that the sample of elderly donor degrades much faster and significantly differs from the younger donor sample.

The content of DeOxyHb in the sample of the elderly donor has been decreasing in the first days of storage. In the sample of the younger donor, the kinetics of the analogous process is more smooth and decreases more than twofold during the first seven days of storage.

All shown above results indicate the presence of individual donor factors influence (the individuality of each donor, age, health condition at the time of donation) on hemoglobin degradation in erythrocytes.

2.3 Discussion

Research investigation has shown that the general trend of hemoglobin degradation during red blood cells storage is expressed by two characteristics. It is deoxyhemoglobin concentration decrease and the oxidized trivalent form ratio growth. It was found that kinetics of these processes varies for RBCs from different donors. It indicates the presence of individual donor factors influence on hemoglobin degradation in RBC.

Recently in transfusion medicine there is an acute problem to assess the donated blood quality. Recent medical statistical researches show that international standards of RBCs

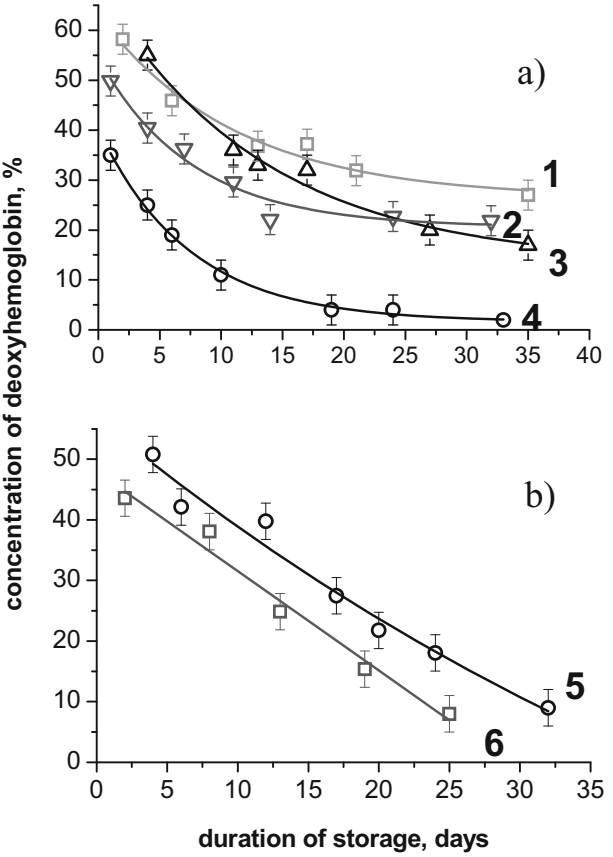

Fig. 5 Deoxyhemoglobin concenration kinetic change during packed red blood cells storage of different donors (1–5): **a**-exponential dependence of deoxyhemoglobin distinct **b**-linear dependence

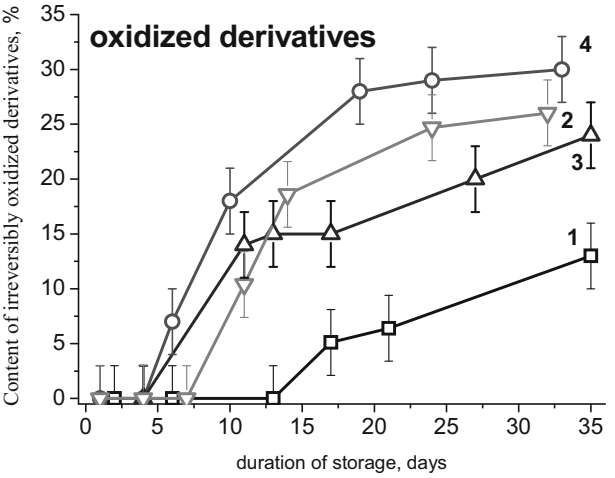

Fig. 6 The change in the trivalent hemoglobin derivatives in the samples of different donors (1–4)

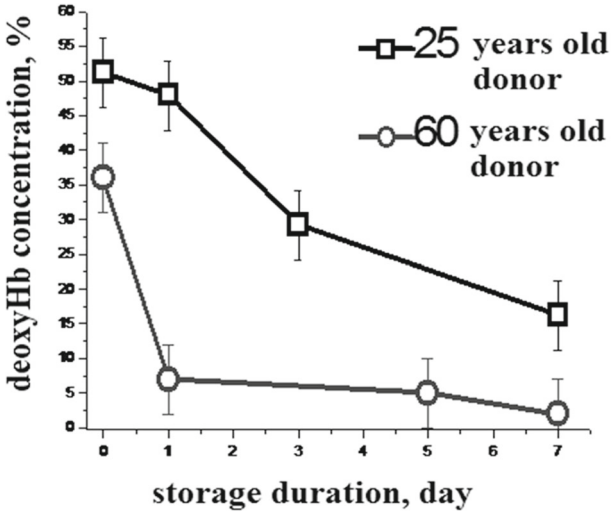

Fig. 7 Kinetic curves for fresh RBC degradation for samples obtained from donors of different ages

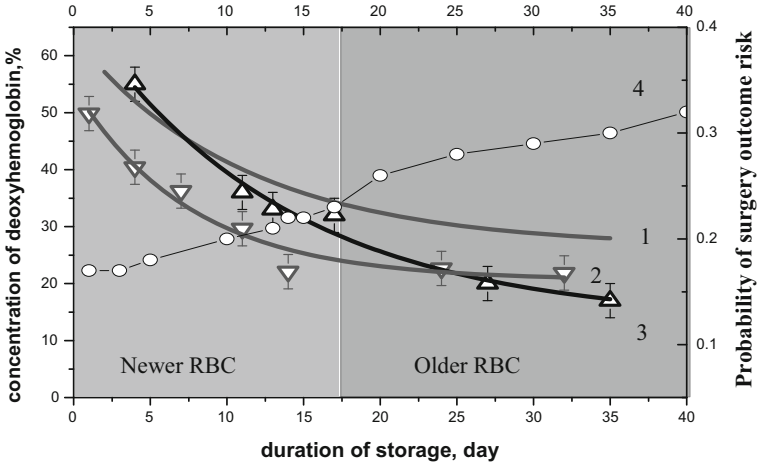

Fig. 8 Comparative analysis of deoxyhemoglobin degradation of RBC material from different donors (1-3) and statistical data (4) of surgery outcome risk increase via RBC?s storage duration from [3]

storage duration limit in clinics are overstated. Indeed, although RBCs are suitable for transfusion, the transfusion efficiency after a certain period of RBCs storage drops sharply. Publication analysis shows that there is a relationship between duration of blood storage and post surgery complications after its transfusion. In the latest American statistical researches (2008-2015) [3] this hypothesis was confirmed. It was found that in the case of a RBC transfusion stored more than 2 weeks patients had post surgery risk increase by 30%. Comparative analysis of deoxyhemoglobin degradation of RBC material from different donors (1-3) and statistical data (4) of surgery risk increase via RBC's storage duration [3] is given in Fig. 8.

The fact that red blood cells lose their functional qualities during storage can be caused by low deoxyhemoglobin concentration and high oxidized trivalent hemoglobin forms ratio. These regularities are observed in the spectra recorded in the middle of a standard storage time. Research investigation showed that the red blood cells storage correspondence is not always respective enough for quality control. According to the significant kinetics differences it is necessary to consider the individual donor characteristics.

3 Conclusion

In our research the general kinetic regularities of hemoglobin's derivatives change during RBC storage are revealed by means of Mössbauer spectroscopy. The study is consistent with confirmed several published statistical data and showed that the use of stored donor's RBC can hardly be guided by the indicated storage period, and specific quality control is necessary for efficiency of transfusion.

It is shown also that in the case of each particular donor, the process of cell degradation can proceed at different rates, and in some cases from the very beginning have a low oxygen capacity. Here we see the possibility of spectroscopic classifying of regular donors by cells shelf life, which may be important for stored cells medical centers.

Based on our result we may suggest an objective criteria for duration of storage for regular donor RBC transfusion suitability obtained from Mössbauer spectral data using DeOxyHb content as a marker.

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