Introduction

During the last twenty years the action of photons within the optical spectral range on blood has become, due to its therapeutic effects, a promising method in transfusiology. Due to its complex composition, the physical and chemical properties of blood have not yet been entirely elucidated. The possibility that blood could easily be collected and preserved for 21-42 days determined the authors' attempt to answer such questions as: the minimum LLLR dose capable of influencing blood qualities without damaging effects, the optimum characteristics of the irradiating source and protocol of irradiation. Because of its complex composition, the physical and chemical properties of blood have not yet been entirely elucidated. Blood irradiation additionally involves a special physical agent, as well as mechanisms of action that are not clearly understood.

Material and Methods: We irradiated blood stored in MacoPharma bags, from seven healthy adult donors. A HeNe laser (6 mW, continuous wave) was used as the source of irradiation. Twenty-four to 48 hours after irradiation blood samples underwent rheological and biochemical analyses in order to establish the morpho-functional status of the red blood cells. Marked effects occurred in the case of effectively received doses of 0.4 and 1.2 J·cm⁻³, suggesting a beneficial action of LLLR on the morphologically functional erythrocytes.

Conclusion: This study demonstrated that blood rejuvenation by the physical method of laser irradiation could be an efficient, simple and economic procedure, and that it needs to be tested on patients who require urgent transfusion. Because the irradiated blood demonstrated a better capacity than the non-irradiated blood, we concluded that LLLR had a revitalizing effect on preserved blood.

Key Words: low-level laser radiation, RBC's viability, rejuvenation, osmotic resistance, viscosity
sition (which defines its optical characteristics) and the preservation conditions. 3,19) Research strategies for studying the stored blood should consider the following salient comments: 1) the fundamental nature of the storage lesion remains unknown; 2) no good surrogate test has ever been found for the performance of viability studies in human volunteers; 3) it is unclear which, if any, of the components of the storage systems are effective in improving the viability of stored RBC after transfusion, and 4) many of the improvements in blood preservation were achieved without the discovery of any new principles. 20)

The study of the LLLR effects on blood is very important in the process of revealing the mechanisms of action of laser radiation on biological tissues, as the blood is permanently composed of a diversity of cells whose membranes contain lipids, sugars and proteins. Blood plasma probably contains the most diverse range of biological products among all tissues, some being in transit to other organs (amino acids, proteins, lipids, hormones, antibodies, adjusting factors), exogenous substances or component substances (clotting and defense enzymatic systems, such as Complement etc.). Some of the substances present in the blood may act as primary acceptors of radiation. 2,3,21)

In the current study we have investigated the integrity and viability of stored RBC after HeNe laser irradiation, as well as the possible beneficial effect of increasing the preservation period. This is a popular research topic in the field at an international level, because increasing the preservation period from 21–42 days to about 60 days would reduce the amount of blood required, and would solve the problems related to rare blood group transfusions. On the other hand, the population's life span is expected to increase by the year 2030; consequently, a necessary supplement of about 25% in blood units for transfusion is envisaged. Producing artificial blood is not easy because of the complexity of the blood, and recent attempts have not been promising. The three main directions of research regarding the preserved blood are: a) defining the intimate processes of cell aging starting from the cellular and molecular mechanisms of aging, aging at finding therapeutic applications by modulating the cell auto destruction program both in vivo and in vitro (in transfusion centers); b) introducing new tests for assessing the quality of the stored blood, and new solutions for ameliorating the conditions of collection and storage of erythrocytes, which have direct implications for prolonging their post-transfusion viability; c) optimizing media for preserving the blood, so that it is able to prolong the viability of preserved RBCs. 1,22,23)

Two common methods of rejuvenating preserved blood are known by now, in respect to the prolongation of life span: 25) – the chemical method, adding inside the blood bags some nutrient principles (phosphoric acid, dextrose, adenine), that support the energetic metabolism of the RBC–oxidative phosphorylation, which maintains the operation of the membrane exchange pumps and the combinative sites where the hemoglobin combines with the oxygen by means of 2, 3-DGP;
– the physical method, periodical stirring and/or oxygenation (the open method), which was abandoned because of its low efficiency and of the contamination risk of blood products.

The new method that we propose for the rejuvenation of preserved blood (i.e. increasing the ratio of young to old RBCs, a process that could possibly be explained by laser radiation enhancing hemolysis of old or non-functional RBCs) using laser blood irradiation is suspected to be viable, efficient, low-cost, non-invasive and pose no blood contamination risk. If this is true, the prospect for performing transfusions using the irradiated blood will be good, thus our method could be used in the treatment of some severe immune or hematological diseases (various types of leukemia). The efficiency of this method was demonstrated when we recorded, after irradiation, a positive modification of some markers of the blood's functional integrity in relation to the non-irradiated samples. The inspiration for using laser irradiation as a physical method of rejuvenation both during preservation and before the transfusion came from recent studies, which have demonstrated the beneficial effect of the LLLR on the membrane of the erythrocytes. 24–28) We studied the variation of some biochemical, rheological and functional blood factors representing markers of the blood functional integrity – depending on the irradiation dose, for different irradiation protocols. We tried to find the optimal doses and mode of administering these doses in order to obtain maximum effects. A lack of cellular structure deterioration was evident as a result of irradiation. The aim of the present research is to prove whether as a result of laser action, it is possible to increase the viability of erythrocytes and get rejuvenation, in order to perform transfusion, as well as prolong the life of the erythrocyte preserved at 4°C.

**Materials and Methods**

**Subjects**

Blood samples were obtained by vein puncture from
seven adult volunteers (male regular blood donors) randomly recruited, after oral informed consent was obtained. All participants were assured of the confidentiality of all data obtained in the study. According to local laws, neither Medical Ethic Committee approval nor written consent is required, unless blood is autotransfused or transfused. Blood samples from donors were referred for the standard blood analyses. Hemolyzed (totally or partially) blood, or blood samples which did not pass the routine analyses were excluded from the study. In order to ensure the request of blinding method, only the coordinator of the study had access to all gathered data.

**Preparation of the samples**

Blood samples were prepared from the whole blood of healthy donors, preserved in MacoPharma bags, and treated with CPD (sodium Citrate as anticoagulant + P, D as preserver). CPD is used to store human blood for periods of approximately 21 days. To have more samples per volunteer, the original bags were separated into smaller bags prior to getting the blood, by using high frequency currents to stick them, each sample being able to collect about 10 ml of blood. VENOJECT test tubes of 7 ml of blood were used as an alternative recipient.

Seventeen small bag samples from the first donor were used, and 20 samples from the second donor (seven bags and 13 test tubes). There was one proof sample from each donor. To determine the normal dynamics of some rheological or biochemical blood parameters during the preservation period, we used five bags from five different donors, whose blood was not irradiated. Our aim was to compare the alterations observed in irradiated preserved blood with the “normal” alterations in the preserved non-irradiated blood. Forty-four samples were used in total (17+20+2+5), including the proof samples. We used 334 experimental values for this study.

**Irradiation procedure**

A He-Ne laser (LG 10, Institute of Physics and Radiation Equipment Technology, Bucharest – Magurele, Romania) was used as source of irradiation (632.8 nm, 6 mW, P/S~75 mW·cm⁻² on the blood surface, continuous wave, 2 mm spot diameter). As in our previous studies on fresh blood, 29,30 where we used HeNe laser and observed maximum effect for about 1.2 J·cm⁻³, doses ranged from 0 (proof sample) to 2 J·cm⁻³. Different irradiation protocols were used for samples from the two donors, as indicated in the legend of figures.

In our studies where we used HeNe laser, due to much larger sample size (10/2cm) against the laser beam size (1/1 mm), we irradiated samples by dots. Thus, only about 10% of erythrocytes were directly irradiated, the other receiving radiation by diffusion. Irradiating samples on both sides, we can consider that the percent of directly irradiated erythrocytes was about 20% or even more, due to the mechanical movements which the samples have undergone. In studies that have used divergent beam sources (LEDs, lamps) it was possible to irradiate directly the entire quantity of red blood cell in the time given. 31)

In calculating the doses, we took into account the energy losses, caused by reflection on the surfaces, strong diffusion into the bag or test tube material, absorption, and other causes. We assessed all these energy losses to be approximately 60% for bags and 35% for test tubes.

Since the hematocrit (HCT), which absorbs the most of radiation, is deposited at the bottom of the container, in order to ensure as homogeneous an irradiation as possible, the sample was turned upside down and stirred in cases when the irradiation of a sample lasted more than five minutes.

**Measurements**

Measurements were made for the following parameters as a function of the irradiation dose:

– from complete blood count (CBC): HCT, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean cell or corpuscular hemoglobin in concentration), using a SERONO Systeme 9020+ computerized hemoanalyzer, (Baker Diagnostics Inc. K&M Company, Torrance, CA);

– ions from blood plasma (K⁺, Na⁺, Ca²⁺, pH), using ILYTE equipment;

– percentage of discocytes or echinocytes, by optical microscopy;

– variation of osmotic resistance (fragility) using the traditional technique with 10 test tubes;

– variation of erythrocyte filtration (Teitel method), and blood viscosity (Preciss viscometer).

In order to obtain a picture of the latency and duration of the LLLR biological effects, the measurements were made 12 to 36 hours after irradiation.

**Data Analysis**

The assessment of the erythrocyte morphology was achieved according to the “morpho-functional criteria of assessing the viability of preserved erythrocytes”. 32-33) We considered variations in the various parameters measured in the experiments as significant when
p<0.085 (for HCT), p<0.09 (MCV and MCH), p<0.055 (MCHC), and p<0.08 (viscosity). As for the remaining parameters in this study, the only available methods were not suitable for statistical approach.

Results

Results are summarized in figures (using Microcal Origin program, version 6.0.), and tables below.

Figs.1 and 2 depict the dynamics of MCV and K⁺ (plasmatic potassium ions) respectively, in the case of the five donors whose blood was not irradiated. A slight trend toward increases of MCV with time, as well as a more significant increase of K⁺ concentration may be seen. As for Na⁺ concentration, variations are in the opposite direction than for K⁺. Table 1 shows that CBC parameters and plasmatic ions remain practically unchanged for doses between 0 and 2 J·cm⁻³, in the

Table 1. CBC parameters and plasmatic ions versus the irradiating dose

<table>
<thead>
<tr>
<th>Crt. no. sample</th>
<th>Dose (J/cm³)</th>
<th>HCT (%)</th>
<th>MCV (µm³)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>K⁺ (mmol/l)</th>
<th>Na⁺ (mmol/l)</th>
<th>pH (mmol/l)</th>
<th>Ca²⁺ (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>0</td>
<td>42.8</td>
<td>91.8</td>
<td>31.5</td>
<td>34.3</td>
<td>19.98</td>
<td>134.9</td>
<td>6.455</td>
<td>0.04</td>
</tr>
<tr>
<td>1 b</td>
<td>0.097</td>
<td>40.1</td>
<td>91.5</td>
<td>31.5</td>
<td>34.4</td>
<td>19.86</td>
<td>138.4</td>
<td>6.395</td>
<td>0.04</td>
</tr>
<tr>
<td>1 c</td>
<td>0.202</td>
<td>41.0</td>
<td>90.4</td>
<td>31.1</td>
<td>34.4</td>
<td>19.99</td>
<td>134.2</td>
<td>6.370</td>
<td>0.04</td>
</tr>
<tr>
<td>1 d</td>
<td>0.374</td>
<td>43.7</td>
<td>91.7</td>
<td>31.7</td>
<td>34.6</td>
<td>20.94</td>
<td>132.6</td>
<td>6.395</td>
<td>0.04</td>
</tr>
<tr>
<td>1 e</td>
<td>0.648</td>
<td>40.5</td>
<td>91.7</td>
<td>32.4</td>
<td>35.3</td>
<td>20.28</td>
<td>133.2</td>
<td>6.405</td>
<td>0.04</td>
</tr>
<tr>
<td>1 f</td>
<td>0.979</td>
<td>41.8</td>
<td>91.5</td>
<td>31.1</td>
<td>34.0</td>
<td>19.67</td>
<td>135.5</td>
<td>6.440</td>
<td>0.04</td>
</tr>
<tr>
<td>1 g</td>
<td>1.188</td>
<td>40.9</td>
<td>92.0</td>
<td>31.9</td>
<td>34.7</td>
<td>19.44</td>
<td>139.0</td>
<td>6.350</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The first donor (little bags)

<table>
<thead>
<tr>
<th>Crt. no. sample</th>
<th>Dose (J/cm³)</th>
<th>HCT (%)</th>
<th>MCV (µm³)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>K⁺ (mmol/l)</th>
<th>Na⁺ (mmol/l)</th>
<th>pH (mmol/l)</th>
<th>Ca²⁺ (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 a</td>
<td>0</td>
<td>45.2</td>
<td>94.2</td>
<td>31.5</td>
<td>33.4</td>
<td>21.25</td>
<td>129.1</td>
<td>6.430</td>
<td>0.04</td>
</tr>
<tr>
<td>2 b</td>
<td>0.47</td>
<td>48.6</td>
<td>93.6</td>
<td>31.0</td>
<td>33.1</td>
<td>21.36</td>
<td>129.7</td>
<td>6.560</td>
<td>0.04</td>
</tr>
<tr>
<td>2 c</td>
<td>0.78</td>
<td>44.6</td>
<td>93.8</td>
<td>31.4</td>
<td>33.4</td>
<td>21.33</td>
<td>131.0</td>
<td>6.420</td>
<td>0.04</td>
</tr>
<tr>
<td>2 d</td>
<td>1.12</td>
<td>46.0</td>
<td>93.8</td>
<td>30.8</td>
<td>32.8</td>
<td>20.93</td>
<td>131.3</td>
<td>6.435</td>
<td>0.04</td>
</tr>
<tr>
<td>2 e</td>
<td>1.38</td>
<td>45.5</td>
<td>94.0</td>
<td>31.4</td>
<td>33.4</td>
<td>20.66</td>
<td>129.7</td>
<td>6.480</td>
<td>0.04</td>
</tr>
<tr>
<td>2 f</td>
<td>1.58</td>
<td>44.1</td>
<td>93.7</td>
<td>32.3</td>
<td>34.5</td>
<td>21.19</td>
<td>136.0</td>
<td>6.370</td>
<td>0.04</td>
</tr>
<tr>
<td>2 g</td>
<td>1.97</td>
<td>45.5</td>
<td>93.9</td>
<td>32.0</td>
<td>34.1</td>
<td>22.32</td>
<td>119.0</td>
<td>6.580</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The second donor (little bags)
case of the two donors whose blood was irradiated. However, we can see that MCV and K⁺ concentration exhibit a slight trend toward decrease for certain dose values. pH was not visibly influenced by laser radiation, while Ca²⁺ ion concentration remained unchanged for all samples.

Morphologically functional erythrocytes (percentage of discocytes in sample) as a function of irradiation dose can be seen in Fig. 3 for the first donor, and percentage of echinocytes in Figs. 4 and 4' for the second one. The irradiation protocol is explained under each figure.

Variation of initial osmotic resistance (fraility) as a function of irradiation dose is presented in Fig 5 for the first donor, and in Figs. 6 and 6' for the second one.

Variation of the erythrocyte filtration (aggregability) as a function of irradiation dose is presented in Fig. 7 for the first donor, where significant fluctuations can be seen. For the second donor significant fluctuations of viscosity can be observed particularly in Fig. 8', in the case of samples irradiated after the preservation

---

**Fig. 3:** Morphology of erythrocytes (the percentage of discocytes) as a function of irradiation dose, and of irradiation protocol (the first donor; the blood was stored in bags for 30 days).

We represented by dotted lines the accepted mean values for the plotted parameter.

To determine if there is a cumulative effect of radiation on erythrocyte morphology, all four samples were finally given the same dose, but in different ways. The first sample received a one-time dose (in the 6th day from the collection), sample 2 has received half a dose in two different days (day 6 and 19), sample 3 received one-third of the total dose in three different days (day 6, 14 and 23) and sample 4 has received a quarter of the dose in four different days (day 6, 12, 19 and 25).
period has expired (curve 2, Fig.8' – starlets).

Discussion

The blood collected in bags with anticoagulant and preserving solutions undergoes, through conservation at 4 to 10°C, a natural process of aging or destruction because of stress. This can be of a physical (collection, transport, refrigeration, and handling) or chemical nature, and manifests itself through modifications of red cell shape, the increase of plasmatic potassium and decrease of sodium, the increase of free hemoglobin and acidosis, and the decrease of viscosity and of osmotic globular resistance. All of these effects can lead to an intense hemolysis even after a few days, accompanied by a consequent decrease of blood viability. Aged red cells show a marked lack of 2, 3-DGP, which enhances the affinity of hemoglobin for oxygen, depending on the preservation period. Thus, the human body cannot benefit from maximum oxyphoric function when transfused with this blood. Complete recovery of the deposits is achieved by circulation within a few days after the transfusion. Though the hematocrit and the hemoglobin clinically increase immediately after transfusion, the effects on the patient are not equally manifest (cyanosis, dyspnoea, pallor

Fig. 5: Initial osmotic resistance as a function of irradiation dose, and of irradiation protocol (the first donor).

We represented by dotted lines the accepted mean values for the plotted parameter. For the four curves in Fig.5 the protocol was similar to that in Fig.3.

Fig. 6: Initial osmotic resistance as a function of irradiation dose (the second donor).

Fig. 6': Initial osmotic resistance as a function of irradiation dose, and of irradiation protocol (the second donor).

For the curves in Figs.6 and 6' the protocol was similar to that in Figs.4 and 4' respectively.
and asthenia are still present). Hence the necessary quantity of transfused blood for ensuring a normal oxyphoric function has to be approximately 10% larger than the estimated quantity (400-450 ml of transfused blood in order to increase hemoglobin by 1g/100 ml of blood).

LLLR may induce, through specific mechanisms, a stimulation of the cellular energetic system that starts with the 2, 3-DGP reloading, so that the red cells once transfused regain full oxyphoric function more rapidly, while the time needed for ameliorating the symptoms, as well as the necessary quantity of blood, diminish. This method of revitalizing the erythrocytes through the recovery of 2, 3-DGP deposits has been called rejuvenation.

CBC. By comparing the results in the case of irradiated bags with those recorded in the case of non-irradiated bags, we can state that laser radiation does not cause significant structural alterations (i.e. hemolysis). We measured only the four mentioned CBC indices because these are specific to the morphological alterations that may occur in the process of blood product preservation.

– HCT and MCH were not significantly influenced by LLLR, meaning that LLLR does not determine structural blood alterations (Table 1). The marked fluctuation in

Fig. 7: Erythrocyte filtration as a function of irradiation dose, and of irradiation protocol (the first donor). We represented by dotted line the accepted mean value for the plotted parameter.
For the four curves in Fig.7 the protocol was similar to that in Fig.3.

Fig. 8: Relative viscosity of the whole blood as a function of irradiation dose (the second donor).

Fig. 8': Relative viscosity of the whole blood as a function of irradiation dose, and of irradiation protocol (the second donor).
For the curves in Figs.8 and 8' the protocol was similar to that in Figs.4 and 4' respectively.
the case of the first donor is due to certain physical factors (temperature about 40°C during irradiation, and additional mechanical movement). In the case of the second donor, the influence of such factors was minimized, due to measures to reduce their influence.

– MCV: the constant values (even a slight decreasing trend) of this parameter as a function of irradiation dose (if MCH and MCHC remain constant) mean that laser radiation acts towards maintaining the shape of cells. This fact, corroborated with the slight increase in time for the same parameter in the case of non-irradiated samples, represents a beneficial effect of laser radiation. This explanation would be valid only if MCH and MCHC were constant. For constant MCHC values an increase of MCV would have meant spherocytosis, which is the first step towards hemolysis, and for reduced MCH the increase of MCV would have meant osmotic spherocytosis: membrane cell damage.

– MCHC: the slight trend towards decreasing in time, in the case of non-irradiated blood samples (five donors) indicates the occurrence of a slight hemolysis, a normal effect in time. The disappearance of this trend for irradiated samples (two donors) means that the HeNe laser radiation acts (under the given irradiation conditions) in the opposite direction of the hemolysis process, having a beneficial influence in this respect. This laser radiation action corresponds to the slight MCV rising trend.

**Plasma ions**

– K⁺: a marked increase with time of the plasmatic potassium concentration was observed in non-irradiated samples, indicating the release of potassium from the cell and the penetration of sodium, which leads to the cell swelling (osmotic equilibrium), a negative effect for the cell, signifying cellular aging and functional alteration. Maintaining constant values of K⁺ concentration in the dose interval between 0 and 2 J·cm⁻³ for the irradiated samples means that laser radiation has no visible effect on K⁺ concentration amelioration. For the irradiation protocol in which doses were applied in one session on the sixth day from collection (table 1, first donor) a slight decrease in K⁺ concentration was found for a dose of about 1-1.2 J·cm⁻³, which represents a beneficial effect of the LLLR (activation of the membrane pumps). The sum of K⁺ and Na⁺ concentrations has to be constant. 23)

– pH: the fact that this parameter was not markedly influenced by laser radiation has the same relevance as in the case of K⁺ (blood modifications are not caused by a marked acidosis).

– the constant values of Ca²⁺ ion concentrations observed in all samples signifies the lack of alterations in the functional status of the membrane channels and also the lack of calcium disequilibrium.

**Morphologically functional erythrocytes (the percentage of discocytes or echinocytes)**

The visible diminishing of the percentage of discocytes in Fig.3 is due to physical factors – the handling and the high temperature during the procedure of irradiation (~40°C, the equivalent of incubation). If we ignored the influence of these factors, the marked variations in the number of morphologically functional erythrocytes, as a function of dose, would indicate a beneficial action of LLLR: the increase of the preservation period of human blood product in transfusional hematology. The maximum effect was found with a dose of about 1.2 J·cm⁻³.

The marked variations of the percentage of echinocytes in Fig.4 indicate an influence of LLLR on the factors which determine the transformation discocyte-echinocyte. Echinocytic (crenated, spiculed) forms occur in red cells stored over 14 days, by alterations in the intra or extracellular environment, and were obtained from outdated human blood. Cells which were discocytes at the time the blood sample was taken may become echinocytes as the plasma ages. 34) According to the literature, 35) the intrinsic mechanism responsible for shifting the discocyte-echinocyte equilibrium in the human erythrocyte is dependent on one or more intracellular or intramembrane factors, which appear to be temperature dependent, and are influenced by the concentration of inorganic phosphor in the medium. The transformation discocyte-echinocyte is reversible, as a function of the intracellular red cell ATP depletion, the only known intrinsic factor to date. The red blood cell shape may be altered by varying different chemical and physical conditions which affect the properties of the membrane and the volume of the cell. 36) The membrane bilayer, as well as the skeleton of the RBC membrane is responsible for the formation of the echinocytic shapes, presumably due to shear deformation of the skeleton. Other studies 37,38) indicate that spectrin (which appears just at the interior of the intact red cell membrane as a filamentous network) plays a role in regulating red cell shape, and in the stability of the lipid bilayer of the red cell membrane. The integrity of this protein has been shown to require red cell metabolism, specifically ATP production and cyclic nucleotide availability. In Fig.4, curve 1 (quadrates), we can see that for the dose of about 0.4 J·cm⁻³ a decrease in percentage of echinocytes appears. This indicates a beneficial effect of laser radiation: it is quite
EFFECTS OF HeNe LASER ON STORED BLOOD 253

possible that, by modulating the ATP production, laser can act as an antiaggregator of spectrin. The fact that in the other case – curve 2 (starlets) – the same beneficial effect was absent, can be explained on the grounds of unsuited environmental conditions and supplementary handling of samples, or other causes.

Variation of initial osmotic resistance (fragility).

By analyzing graphs in Figs.5, 6 and 6′ the conclusion can be drawn that LLLR does not have a significant influence on this parameter. Some fluctuations occurring from one sample to another one are normal, these being related to specific phenomena concerning preservation, such as the change of the surface–volume ratio of the red blood cells, or the spherocytosis mentioned when we discussed the MCV alterations for a constant MCHC. Fluctuations in Fig.5 and Fig.6′, like in the case of discocyte percentage (Fig.3), indicate the occurrence of structural and functional changes of the erythrocyte membrane (aging).

Variation of the erythrocyte filtration (aggregability)

Marked fluctuations of erythrocyte filtration or viscosity, mainly observed in Fig.8′ for the samples irradiated after the preservation period time limit (curve 2, Fig.8′ – starlets) indicate the occurrence of structural and functional alterations of the erythrocyte membrane. LLLR influence is significant mainly for doses between 1 and 1.2 J·cm⁻³, but also in the interval 0.2-0.6 J·cm⁻³. In the case of the variation of erythrocyte filtration fluctuations in Fig.7 seem to be due to the environmental conditions to which the respective samples were subjected. However, we consider LLLR influence to be relevant to both erythrocyte filtration and blood viscosity. The results were not influenced by some causes such as: agglutination, self-antibodies, cryo-antigens etc.

Conclusions

In this study we investigated variations in four CBC parameters: plasma ion concentration, integrity of the morphologically functional cells, osmotic resistance, and erythrocyte filtration (or viscosity) for irradiated blood, as a function of irradiation dose and of the irradiation protocol. We also explored the dynamics of some CBC parameters and plasma ions concentration in the non-irradiated blood.

In the case of CBC parameters we mainly followed MCV variation, corroborated with MCHC. The conclusion has been that laser radiation acts in the direction of maintaining the shape of cells, without leading to negative phenomena like spherocytes and hemolysis, which normally occur in non-irradiated blood during the preservation period.

Dose values of under 0.5 J·cm⁻³ stimulated preserved blood erythrocyte aging, leading to low viability. For doses of 1.2 J·cm⁻³ we noticed that functions and structures were maintained between acceptable physiological limits (we had in mind especially the functional status of erythrocyte rather than phenomena at the hemoglobin level).

In the case of parameters that were significantly modified by LLLR action, these were generally positively influenced, usually with maximum effects for doses ranging between 0.4-0.6 J·cm⁻³ and 1-1.2 J·cm⁻³.

The results indicate that irradiation effects differ, to a certain extent, depending on the irradiation protocol (number of days from collection, dose given in one session or in several sessions).

Important alterations of the erythrocyte membrane occurred in the first donor’s blood morphological structure (spherocytes and acancytosis = echinocytes), that is its aging, because the sensitivity of red cells was increased due to certain physical factors (temperature, additional mechanical stirring). In the case of the second donor, when the influence of the above-mentioned factors was considerably reduced, the aging effect was reduced.

The practical recommendations that can be derived from the conclusions on the functional status of erythrocyte when analyzing the appropriate graphs are the following:
- high temperature during irradiation is harmful (Fig.3);
- optimal irradiation doses in order to obtain some beneficial effects is around the value of 1.2 J·cm⁻³;
- the best results occur in the case of blood preserved in bags, in contrast to vacuum test tubes;
- It is recommended that the irradiation was done no more than a week from the collection of blood, and give the dose in one session rather than in several sessions (Fig.3, curve 1).

The new method that we propose for the rejuvenation of preserved blood enjoys several advantages: low cost, non-noxious or harmful, easy to implement, closed system procedure, and few drawbacks. Consequently, the method of laser irradiation of preserved blood bags could be part of the current transfusion practice before long.

On the basis of the experimental data we have gathered in this study, we consider that the LLLR revi-
talizing-stimulating effect (rejuvenation) on preserved blood is relevant. It follows that clinicians should study this method by exploring the effects on patients of transfusing irradiated blood (more rapid disappearance of cyanosis, dyspnoea, asthenia) in contrast to the patients who received non-irradiated blood or blood rejuvenated by other methods. Using other more precise methods of investigation (flow cytometry, scanning electron microscopy etc.) will allow us to better assessment for the effects of LLLR on stored blood.

References

24: Cui Y, Guo Z, Zhao Y et al. Reactive effect of low intensity He-Ne laser upon damaged ultrastructure of human erythrocyte membrane in Fenton system available at www.jstage.jst.go.jp/browse/islsm


This work was done at the Centre of Transfusion Hematology of the Army.
Source of support: The Center of Transfusion Hematology of the Army for the equipment