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The Osmotic Fragility of Human Erythrocytes is Inhibited by Laser Irradiation

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Abstract: In this study we investigated the influence of green laser irradiation (532 nm, 30 mW, 31.7 J/cm^2) on the membrane integrity of human erythrocytes and compared the results with the effect of infrared laser irradiation (810 nm, 50 mW, 31.3 J/cm^2). To evaluate the membrane integrity of erythrocytes, one clinical parameter, the osmotic fragility, was investigated. We observed a decrease in osmotic fragility of the erythrocytes after irradiation by the green laser light as well as by the infrared laser compared to non-irradiated controls.

Keywords: Laser irradiation, osmotic fragility, erythrocytes.

1. Introduction

Disease transmission is one of serious complications of the blood transfusion. Stored blood and blood products may contain virus-infected cells which cannot be detected because of the lack of suitable screening tests. As well, some Gram-negative bacteria strains are capable of growing at 4 C, the storage temperature. The risk of the infection is highest for chronically transfused patients [1]. This risk has been reduced consideably for coagulating factor concentrates by the application of virudical procedures. These procedures, however, are not applicable to blood products containing cell components, due to the fragility of these cells. It was suggested that photosterilization of blood (application of photosensitizer and subsequent irradiation with a laser) might protect the patients against the risk of transmitting pathogens [2].

However, such a procedure might change some other functions of blood. A positive effect of low-energy laser irradiation on regeneration has been found in various tissues, such as skin [3], bone [4], nerves [5] and skeletal muscle [6].

In this study we investigated the influence of green laser irradiation on the membrane integrity of human erythrocytes and compared the results with the effect of infrared laser irradiation which is used for the improvement of rheological properties of blood. To evaluate the membrane integrity of erythrocytes, one clinical parameter, the osmotic fragility, was investigated.

2. Materials and Methods

Human blood was obtained from the Clinic of Hematology and Transfusiology, Cyril and Method's Medical Hospital, Bratislava. Blood from healthy adult donors contained 3.2 % sodium citrate as an anticoagulant. The blood was partly depleted both of plasma and trombocytes and it was used at a hematocrit of 56.81 ± 4.62 %. For irradiation of the blood samples we used Nd:YAG Green laser, 532 nm, 30 mW, 31.7 J/cm^2 from Raise Electronics, Taiwan or infrared Therapy Laser CTL 1106 Mx, 810 nm, max. 400 mW, 31.3 J/cm^2 from Laser Instruments, Warsaw. Controls were kept in dark under the same conditions. For the osmotic fragility test, blood sample was added to sodium chloride solutions in the concentration range of 0 to 150 mmol/l. The solutions were buffered with phosphate saline (1.9 mmol/l NaH_2PO_4, 8.1 mmol/l Na_2HPO_4, pH 7.4). Thirty minutes after the incubation of human blood in sodium chloride solution the suspension was centrifuged at 500 g for 20 min. Obtained supernatant was examined spectrophotometrically (Specord M40, Carl Zeiss, Germany). The amount of the released hemoglobin in the supernatant was determined by examining the absorbance at 541 nm, which is one of the hemoglobin spectrum maxima.

The osmotic fragility curves were obtained as a dependence of the concentration of NaCl on the absorbance of hemoglobin at 541 nm. All the measurements were carried out at room temperature 20 ± 2 C. The results were expressed as the mean ± standard error of the mean (SEM). Student's paired *t*-test was used for the statistical analysis.

3. Results

We studied the influence of 532 nm and 810 nm laser irradiation on osmotic fragility of human erythrocytes. In paired experiments, the osmotic fragility curves were measured for the non-irradiated control sample and for the irradiated one. An example of the obtained data for one blood sample is shown in Fig. 1(A) for the non-irradiated and in Fig. 1(B) for the irradiated samples. The observed shape of the curve is typical for the osmotic fragility measurements. We fitted the obtained data by the sigmoidal function:

$$A \quad \frac{A_{\max} \quad A_{\min}}{1 \quad e^{\frac{c \quad c_{50}}{dc}}} \quad A_{\min} \ , \tag{1}$$

were c is the NaCl concentration, A is the absorbance at 541 nm. In distilled water the erythrocytes underwent swelling and subsequently the ruptured erythrocyte membranes released hemoglobin, which was detected as the maximal absorbance value A_{max}, A_{min} is a minimal absorbance value of hemoglobin present in the blood sample in isotonic solution (150 mmol/l NaCl) which has no effect on the integrity of the erythrocyte membranes.

To quantify the degree of osmotic fragility the inflection point, c_{50}, of the sigmoidal curves for non-irradiated and irradiated blood samples was determined. The inflection point corresponds to the concentration of sodium chloride where we observed the absorbance of hemoglobin equal to half of the maximal absorbance value. We obtained a significant ($p < 0.001$) decrease in the c50 values from 72.25 ± 0.69 mmol/l of NaCl for the irradiated blood samples (532 nm, 30 mW, 31.7 J/cm^2) to 70.74 ± 0.61 mmol/l of NaCl for the control sam-

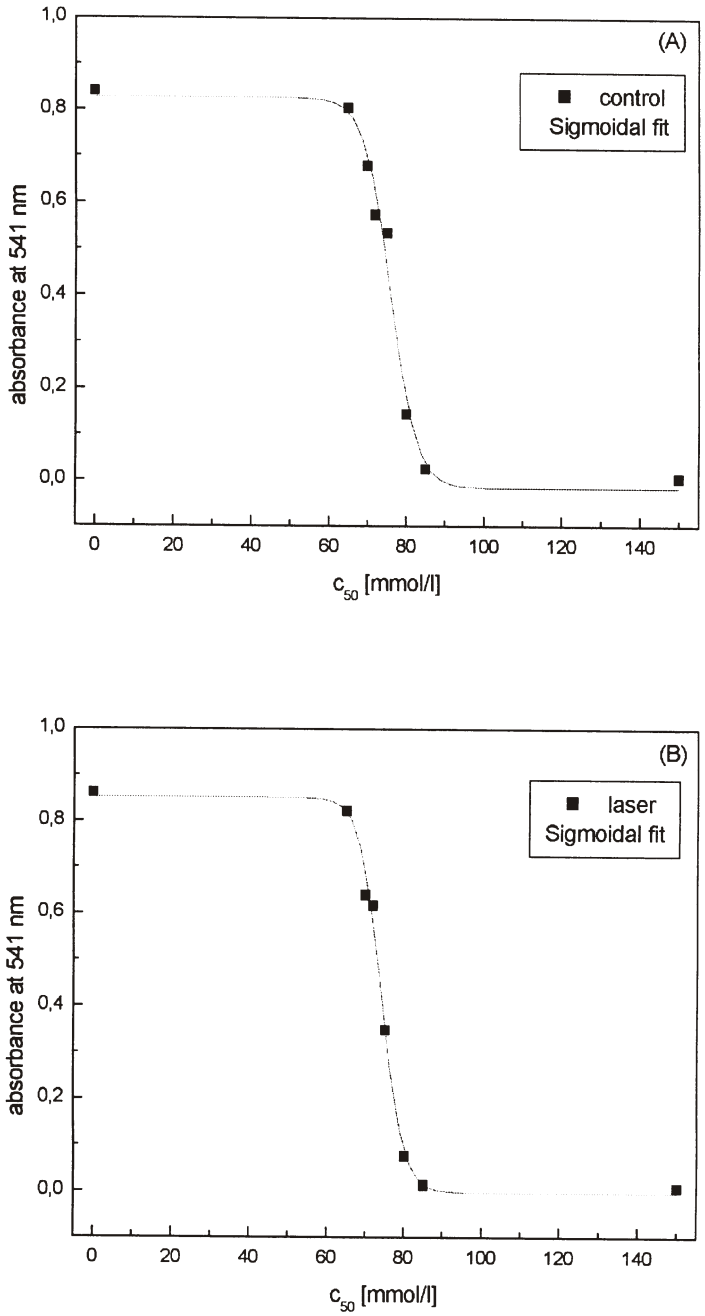

Fig. 1. Osmotic fragility curve for an example of (A) control blood sample and (B) 532 nm laser irradiated blood, both with a sigmoidal fit according to Eq. (1).

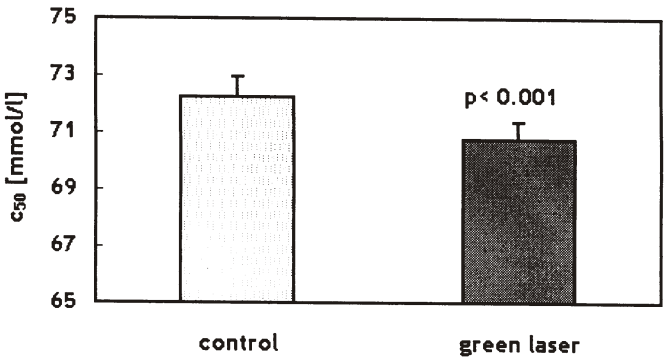

Fig. 2. Effect of green laser light (532 nm, 30 mW, 31.7 J/cm^2) on osmotic fragility of human erythrocytes expressed as c_{50} values. The data are expressed as mean ±SEM of 9 independent experiments.

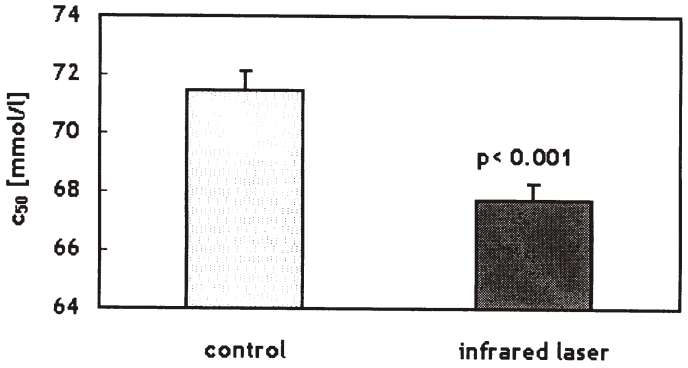

Fig. 3. Effect of infrared laser light (810 nm, 50 mW, 31.3 J/cm^2) on osmotic fragility of human erythrocytes expressed as c_{50} values. The data are expressed as mean ±SEM of 9 independent experiments.

ples. A decrease in osmotic fragility of the erythrocytes after irradiation by the green laser light is shown in Fig. 2. We observed also a significant ($p < 0.001$) decrease in the c_{50} value from 71.45 ± 0.65 mmol/l of NaCl for the control sample to 67.68 ± 0.56 mmol/l of NaCl for the irradiated blood sample by 810 nm, 50 mW, 31.3 J/cm^2 laser light. For the infrared laser, a decrease in the osmotic fragility was observed as shown in Fig. 3.

4. Discussion

In this study we obtained the c_{50} values which were in the range of 50–75 mmol/l of sodium chloride corresponding to the values in the blood of healthy persons [7]. Laser irradiation of the human blood samples, both green (Fig. 2) and infrared lasers (Fig. 3), decreased the c_{50} values when comparing to the non-irradiated ones. That indicates a decrease in the osmotic fragility of the erythrocytes. The main indicator of the susceptibility of erythrocytes to the osmotic pressure is change in their shape, which is dependent on the volume, surface area and functional state of the erythrocyte membrane. Komorowska et

al. [8] shown, that 10 min of irradiation (700–2000 nm, 6.9 mW/m^2) transformed the shape of erythrocyte cells from discoechinocytes to echinocytes.

Iijima et al. revealed that the low power He-Ne laser irradiation produces a protective effect on erythrocyte membranes, reducing osmotic fragility and stabilizing the cell membrane [9]. It is assumed that the laser irradiation inhibits the osmotic fragility of human erythrocytes.

Cooling of blood prevents erythrocyte damage after 630 nm dye laser irradiation because the observed blood damage caused by irradiation (210 mW/cm^2) was mainly caused by undesired heating up of the sample [10].

Considering biological action of the laser light irradiation, it should be noticed that only the absorbed light energy can affect the biological object. Consequently, the primary question is what type of molecules are present in the investigated sample and are able to absorb the laser light. In human erythrocytes, green light (532 nm) can be absorbed by heme-containing compounds, especially by hemoglobin, but also by the enzymes such as catalase and peroxidase. Light in the range of red and infrared wavelengths is absorbed by enzymes with porfyrin structure like catalase, cytochrome or superoxide dismutase. In general, after absorption of photon and promotion of electrically excited states, energy transfer and energy transformation initiate a cascade of biochemical reactions and signaling pathways [11] that can lead to a measurable biological effect. Unfortunately, these processes are not yet understood although some possible mechanisms of light action on cells and on cellular signaling have been discussed recently [12].

A release of the hemoglobin into the supernatant indicates damage to the erythrocyte membranes or even complete disintegration of the membranes. We observed a significant increase in the osmotic resistance of the irradiated sample when comparing to the control one. The laser irradiation per se did not have any harmful effect on the physical state of the membranes.

We have assumed it might even trigger some biostimulation effect(s) on the erythrocyte membranes. Precise mechanisms of the biostimulation remain obscure at the time being.

It can be concluded that low level laser irradiation does not have any negative effects on the integrity of human erythrocyte membrane.

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