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LASER-INDUCED CHANGES IN SUPEROXIDE DISMUTASE ACTIVITY AND LIPID PEROXIDATION OF BLOOD *IN VITRO*

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The influence of red and infra-red low intensity laser irradiation (LILI) at different doses on superoxide dismutase (SOD) and catalase (CAT) activities and lipid peroxidation (LP) processes in the rabbit blood was studied. LILI at energy densities of 0.8–21.6 J/cm^2 was shown to activate SOD and inhibite LP processes in the rabbit blood *in vitro*. These data can explain the positive medical effects of laser blood irradiation.

INTRODUCTION

In recent years the treatment of blood with low intensity laser irradiation (LILI) has become widely useful in a variety of clinical applications because of its anti-inflammatory, biostimulative, immune-stimulatory etc. action. Up to now the various effects of He-Ne laser on blood cells either *in vitro* or *in vivo* have been reported. Unfortunately, the available data on stimulating and suppressing doses of LILI remains to be contradictory. Thought the stimulating action of laser light was shown for the narrow interval of irradiation doses and it has large variability (Karu, 1999).

The membrane components of blood cells and molecular components of blood plasma are considered to absorb laser light. According to the literature data, the LILI causes the structural modification of a membrane surface of erythrocytes (Karu, 1999; Iton, Murakami, Orihashi, Sueda & Matsuura, 1996), leukocytes (Klebanov, Teselkin, Babenkova, Bashkueva, Modestova, Steklova & Vladimirov, 1997) and platelets (Olban, Wachowicz, Koter & Bryszewska, 1998) followed by changes in their functional activity. A hypothesis of free radical mechanisms of the stimulating action of LILI suggests that light absorption induces the formation of primary radicals that triggers the subsequent free radical reactions including the lipid peroxidation (Klebanov *et al.*, 1997, Chichuk, Strashkevich & Klebanov, 1999). It is supposed that a photoacceptor of laser radiation may be enzymes of antioxidant defence system containing Fe and Cu, for example, superoxide dismutase (SOD) and catalase (CAT) (Gorbatenkova, Vladimirov, Paramonov & Azizova, 1989). Usually light in red and infra-red regions of a spectrum is used for laser blood irradiation (Volotovskaya & Ulaschik, 2000). Despite the numerous reports on laser cellular effects, the mechanisms of interaction of LILI with blood and its components are still far from being clear.

The aim of the present work was to determine the doses of red and infra-red laser light influencing SOD and CAT activities and effecting lipid peroxidation (LP) in the rabbit blood *in vitro*.

MATERIALS AND METHODS

Heparinized fresh blood taken from central auricular vein of the rabbits was used.

The experiments were carried out using both whole blood and erythrocytes. Red blood cells were separated from plasma by centrifugation of blood at 3000g for 15 min, washed three times with 155 mM NaCl solution and resuspended in this solution at the 10% hematocrit. Blood was diluted with 155 mM NaCl solution at the ratio of 1:5.

Irradiation conditions. The Lazurit-3M apparatus (Belarus), containing the He-Ne laser (λ = 630 nm) and infra-red laser (λ = 770–860 nm) was used. Diameter of a light spot was 3 mm.

The power density values measured with a power meter PMA 2100 (Solar, USA) were 80

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W/m^2 for red and 90 W/m^2 for infra-red laser light. Exposure times were 100, 500, 1000 and 2400 s. The radiation doses calculated according to Kitchen and Partridge (1991) were 0.8, 4, 8 and 19.2 J/cm^2 for red and 0.9, 4.5, 9 and 21.6 J/cm^2 for infra-red laser.

2 ml of diluted blood or 2 ml of 10% erythrocyte suspension were exposed to laser irradiation at the above mentioned energy densities at 20 ± 2°C and under permanent shaking.

SOD activity was determined by an indirect method based on the use of reaction of superoxide-dependent oxidation of quercetin, proceeding in alkaline medium in the presence of tetramethylenediamine (Kostyuk & Potapovitch, 1989).

In order to treat erythrocytes with *tert*-butyl hydroperoxide (t-BHP) the 10% suspension of erythrocytes (in 155 mM NaCl solution) or blood diluted with the same solution at a ratio of 1:5 were incubated for 30 min at 37°C in the presence of 0.75 mM t-BHP. It is known that erythrocyte hemolysis does not occur at such t-BHP concentration.

The efficiency of lipid peroxidation was measured by the thiobarbituric acid technique. The concentration of thiobarbituric acid-reactive substances (TBARS) in supernatants was estimated spectrophotometrically (Stocks & Dormandy, 1971).

CAT activity was determined according to the method described by Korolyuk, Ivanova, Maiorova and Tokarev (1985).

Spectrophotometric measurements were performed using a Specord M40 spectrophotometer (Zeiss, Germany).

The results were subjected to statistical evaluation using the Student's "t" test. The data are given as means ± SD.

RESULTS AND DISCUSSION

It was shown that after *red laser* irradiation of *whole blood* a dose-dependent increase in SOD activity was observed in 80% of cases. The data presented on Fig. 1A demonstrate that there was a small increase in SOD activity level by 10–15% at doses 0.8 and 4 J/cm^2, whereas at 8 and 19.2 J/cm^2 an increase in SOD activity was close to 30–40% above the control level. At the same time the LP

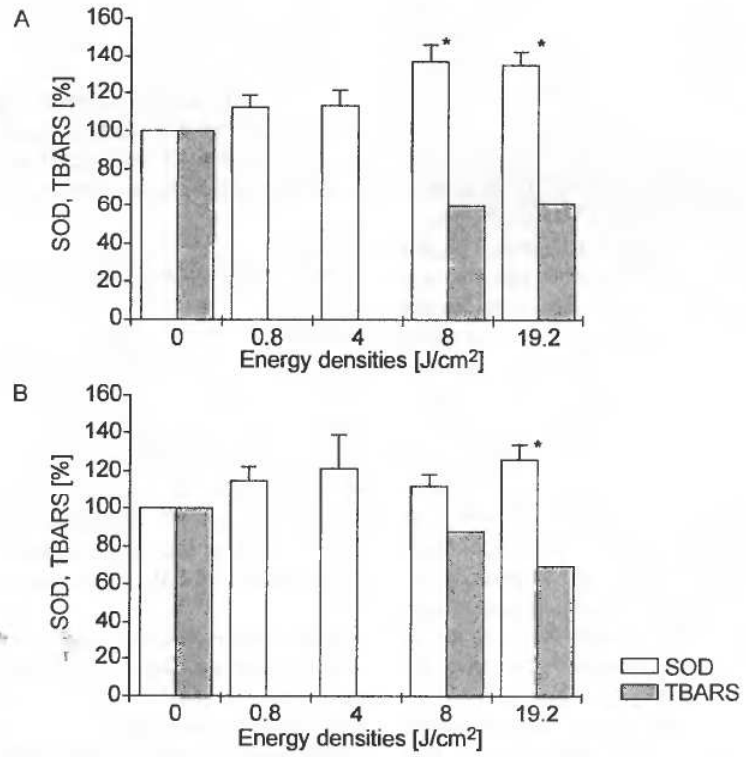

Fig. 1. Changes in SOD activity and TBARS level of whole blood (A) and erythrocytes (B) after exposure to He-Ne laser λ = 630 nm at energy densities of 0.8, 4, 8 and 19.2 J/cm^2. The SOD activity and TBARS level in the control samples were taken as 100% (n = 7). *statistically significant differences, $P < 0.05$

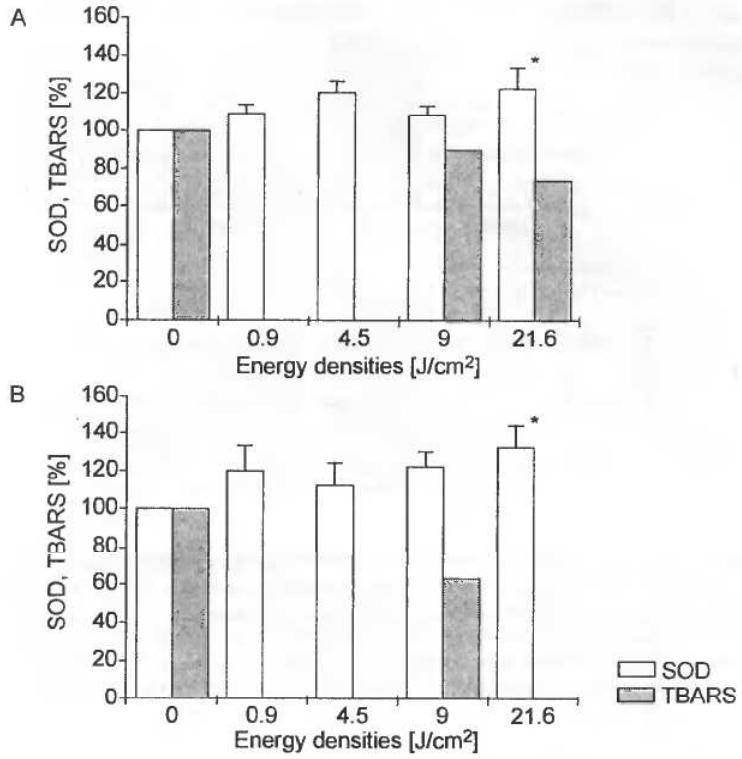

Fig. 2. Changes in SOD activity and TBARS level of whole blood (A) and erythrocytes (B) after exposure to infra-red laser λ = 770–860 nm at energy densities of 0.9, 4.5, 9 and 21.6 J/cm^2. The SOD activity and TBARS level in the control samples were taken as 100% (n = 7). *statistically significant differences, $P < 0.05$

processes in blood were suppressed. The level of TBARS was reduced by 30–40% at the doses of 8 and 19.2 J/cm^2, respectively.

Exposure of blood to the *infra-red* laser irradiation under the same conditions led to the similar results. However, the activation of SOD and reduction of TBARS were less pronounced. The maximum increase in SOD activity by 20–30% and reduction of TBARS formation by 20–30% were observed at 21.6 J/cm^2 (Fig. 2A).

The results of similar experiments, which have been carried out on erythrocytes, are presented in Fig. 1B and 2B. The exposure of 10% rabbit erythrocyte suspension to the red and infra-red laser irradiation resulted in an increase in SOD activity and a decrease in TBARS formation as well as in the case of blood irradiation. It means that the erythrocytes but not other cells or blood plasma contribute significantly to the activation of SOD after blood irradiation.

The protective antioxidant action of LILI effected also the t-BHP-induced lipid peroxidation processes in blood and in erythrocytes. The data presented in Fig. 3 demonstrate the TBARS levels in blood (A) and erythrocytes (B) treated with t-BHP. The red laser irradiation of erythrocytes and blood at the energy densities of 8 J/cm^2 and infra-red at 9 J/cm^2 reduced the level of subsequent t-BHP-induced LP processes by 20–50%.

It should be also noted that red and infra-red laser light has an inhibitory effect on the parameters studied in rabbits. The SOD activity was reduced by 20–30% and in parallel TBARS level was raised by 20–40% (not shown). Unfortunately no explanations for the inhibitory action of LILI has been published.

As it can be seen in Fig. 4 the exposure of 10 % erythrocyte suspension to red laser light (0.8, 4, 8 and 19.2 J/cm^2) and to infra-red laser light (0.9, 4.5, 9 and 21.6 J/cm^2) did not significantly influence the catalase activity in rabbit erythrocytes.

The obtained results confirm the hypothesis that LILI of different wavelength characteristics (red and infra-red light ranges) has a stimulating effect on antioxidant defence systems in red blood cells. The energy densities of 0.8–19.2 J/cm^2 of red and 0.9–21.6 J/cm^2 of infra-red laser stimulated SOD activity and inhibited LP processes which can explain positive medical effects of laser blood irradiation. The activation of SOD was observed in

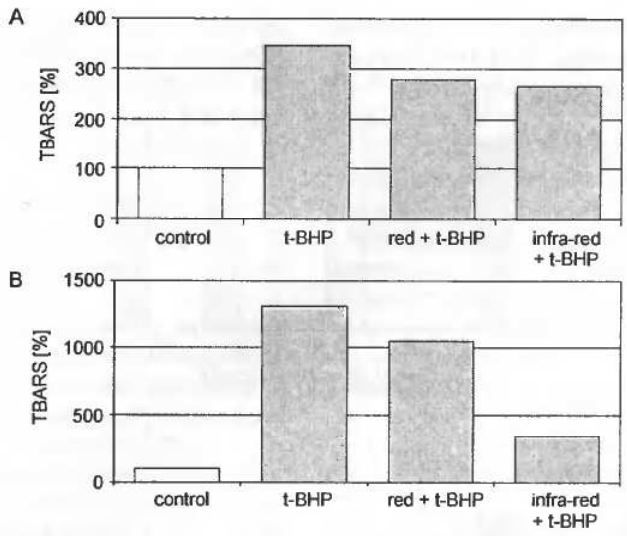

Fig. 3. The influence of low intensity laser irradiation on t-BHP induced lipid peroxidation processes in blood (A) and erythrocytes (B) of rabbits: t-BHP and TBARS levels after samples incubation for 30 min at 37°C in the presence of 0.75 mM t-BHP; red + t-BHP, TBARS levels after exposure of samples to red laser irradiation, $\lambda = 630$ nm at the energy densitiy of 8 J/cm^2 followed by incubation at 37°C for 30 min in the presence of 0.75 mM t-BHP; infra-red + t-BHP and TBARS levels after exposure of samples to infra-red laser irradiation, $\lambda = 770$–860 nm at the energy densitiy of 9 J/cm^2 followed by incubation at 37°C for 30 min in the presence of 0.75 mM t-BHP. The TBARS level in the control samples was taken as 100%

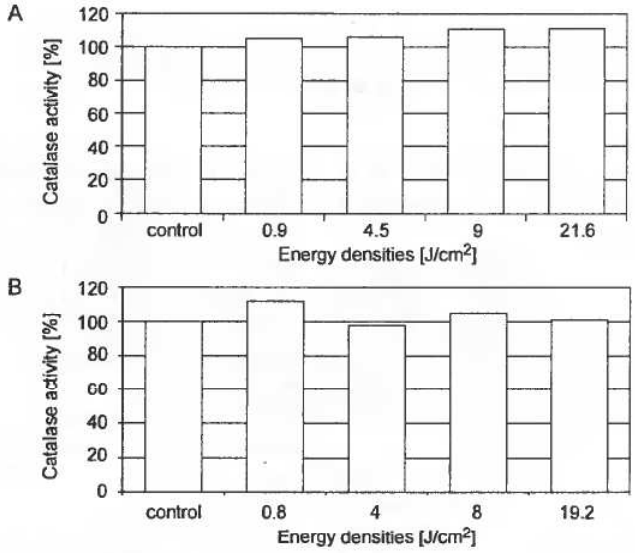

Fig. 4. Catalase activity of erythrocytes after the infrared (A) and red (B) laser irradiation. The catalase activity in the control samples was taken as 100% (n = 4)

80% of our experiments for red and infra-red radiation. Contrary to SOD and LP, the catalase activity was not sensitive to the types of laser irradiation employed. The presented data indicate the necessity of search for sensitive criteria to select the appropriate doses for LILI medical application.

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