REFERENCES


CORRELATION BETWEEN CELL COMPOSITION OF THE SPLEEN AND CHANGES IN SPLENOCYTE CHEMILUMINESCENCE AFTER LASER IRRADIATION

T. N. Andreichuk, T. I. Karu, and T. P. Ryabykh

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Low-intensity laser therapy (He—Ne laser, \( \lambda = 632.8 \text{ nm} \), and various semiconductor lasers, \( \lambda = 800-900 \text{ nm} \)) has been successfully used in the treatment of diseases connected with various kinds of inflammatory processes [4, 6]. In particular, laser irradiation leads to more rapid healing of wounds, trophic ulcers, and burns. During wound healing an active role is played by neutrophils and macrophages, which rid the injured part of infection, and also by lymphocytes and fibroblasts. During phagocytosis active forms of oxygen (AFO) are formed: the superoxide anion-radical, hydroxyl radical, hydrogen peroxide, etc., which perform a bactericidal function [2, 8]. It has also been shown that certain populations of lymphocytes [9], epidermal cells [5], and others have the ability to generate AFO. The appearance of AFO can be recorded by measuring the chemiluminescence (Chl) which accompanies this process, and which is magnified many times over in the presence of luminol.

Intact cells possess spontaneous chemiluminescence (SChl), which reflects the initial state of metabolic processes in the cell [2]. Under the influence of various stimuli, the chemiluminescent response may alter, i.e., the quantity of AFO generated by cells can increase or decrease [2]. The writers showed previously [3, 7] that irradiation by low-intensity red light (\( \lambda = 632.8 \text{ nm} \)) within the dose range 100-300 \( \text{J/m}^2 \) stimulates AFO formation in mouse spleen cells. The aim of this investigation was to study the action of infrared laser radiation on chemiluminescence of splenocytes and to study how the effects of irradiation depend on the cell composition of the irradiated suspension.
Fig. 1. Kinetic curves of chemiluminescence of mouse splenocytes. Abscissa, time (in min); ordinate, Chl (in pulses/10 sec). 1) SChl, 2) Chl after irradiation.

Fig. 2. Correlation fields and regression lines. Abscissa: a) percentage of neutrophils, b) percentage of plasma cells, c) percentage of lymphocytes; ordinate, changes in chemiluminescence of mouse splenocytes (in %) after laser irradiation.

EXPERIMENTAL METHOD

Male A/Sn mice aged 1.5-2 months (13 experiments) and 8-9 months (16 experiments) were used. The mice were obtained from the "Stolbovaya" Nursery and were kept in the animal house under standard conditions. In each experiment a preparation obtained from one mouse was used. The mice were killed by cervical dislocation and the spleen was removed and weighed. The mass of the spleen in the group of young mice varied from 60 to 94 mg and in the group of old mice from 62 to 220 mg. A suspension of splenocytes was prepared as described in [3]. The number of cells was counted in a Goryaev's chamber. Part of the cell suspension was adjusted to a final concentration of $4 \times 10^6$ cells/ml and this was subsequently used in the experiments. The remaining cells were sedimented by centrifugation, one drop of bovine serum was added to the residue, and a film was made, fixed with methyl alcohol, and stained by the Romanovsky-Giemsa method. In each film 1000 cells were identified.

The cell suspension was irradiated with radiation from a "Biotherapy 3ML" GaAlAs laser (Great Britain) ($\lambda = 820$ nm) with pulse repetition frequency of 292 Hz. The power of the laser beam was reduced to 1.2 mW by means of an NS-9 filter. Irradiation was carried out in 96-well round-bottomed plates. Into a well with diameter 5 mm at the bottom 100 $\mu$l of the cell suspension was introduced and irradiated for 18 days from above at a distance of 1 cm from the surface of the suspension; under these circumstances the dose of irradiation was $1.1 \times 10^3$ J/m$^2$. Under these conditions the laser beam completely covered the surface of the irradiated well. Chemiluminescence was measured for 40 min on a "Biolumat" instrument (model LB 9500, "Berthold," Germany). The irradiated cells, and also intact cells, in which spontaneous chemiluminescence (SChl) was determined, were transferred into standard plastic test tubes of the instrument, containing 100 $\mu$l of a 20 mM solution of luminol ("Serva") in sodium-phosphate buffer, pH 7.2, and 100 $\mu$l of medium 199. Kinetic curves were plotted from the results (Fig. 1) and for correlation analysis values of Chl 18 min after irradiation were used. The statistical analysis was carried out on an IBM PC/AT personal computer, using the Statgraf program package.