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

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KEY WORDS: laser radiation; semiconductor laser; mouse splenocytes; chemiluminescence

Low-intensity laser therapy (He–Ne laser, λ = 632.8 nm, and various semiconductor lasers, λ = 800-900 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 (λ = 632.8 nm) within the dose range 100-300 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.

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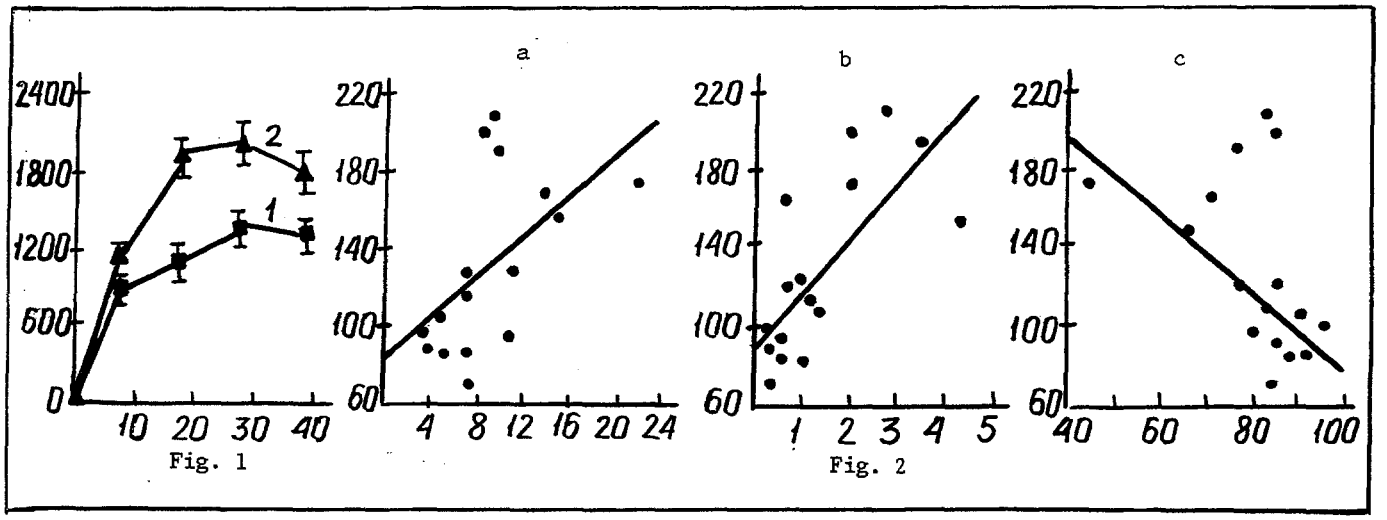

Fig. 1. Kinetic curves of chemiluminescence of mouse splenocytes. Abscissa, time (in min); ordinate, Chl (in pulses/10 sec). 1) SChl, 2) Chl after irradiation.

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 $4 \cdot 10^6$ 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) (λ = 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 μl 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 $1.1 \cdot 10^3$ 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 μl of a 20 mM solution of luminol ("Serva") in sodium–phosphate buffer, pH 7.2, and 100 μl 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.

TABLE 1. Coefficients of Correlation (r) between Effect of Irradiation of A/Sn Mouse Spleen Cell Suspensions and Relative Percentages of Different Types of Cells

Types of cells	Coefficients of correlation, age of mice (months)		
	1,5—2 n=13	8—9 n=16	Total, n = 29
Lymphocytes	—0,417	—0,507*	—0,590***
Plasma cells	0,272	0,748***	0,743***
Monocytes and macrophages	,031	—0,223	—0,131
Myelocytes and metamyelocytes	0,274	0,463	0,507**
Neutrophils	0,341	0,519*	0,650***
Eosinophils	0,668*	0,071	0,179
Other forms	0,374	0,318	0,177

Legend. n) Number of animals in group. Statistically significant correlations: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

EXPERIMENTAL RESULTS

In the first series of experiments A/Sn mice aged 8-9 months were used. The results of 16 experiments showed that irradiation under standard conditions had different effects on cell chemiluminescence. In eight cases irradiation caused an increase in Chl (Fig. 1), up to 120-200%, but in three cases a decrease of Chl by 15-30% was observed, and in five experiments irradiation had no effect on SChl.

For comparison, in the second series of experiments younger A/Sn mice aged 1.5-2 months were used (13 experiments). Irradiation under the same conditions as in the first series of experiments caused either inhibition of SChl or no effect.

Analysis of the cell composition of the splenocyte suspensions revealed that in some cases an increase in the fraction of granulocytes, monocytes and plasma cells was observed in 9-month-old mice compared with young mice. For instance, the fraction of neutrophils in films prepared from the spleen cells of 9-month-old mice varied from 3.4 to 20%, and plasma cells accounted for 0.1-4.1%; for mice aged 1.5 months, however, these values were 1.1-7.2% and 0.2-1% respectively.

To discover how the effect of irradiation (i.e., the change in Chl, expressed as a percentage of the numbers of different types of cells present in the suspension) depends on irradiation, correlation analysis was carried out (Table 1). For A/Sn mice (8-9 months old) positive correlation was found between the effect of irradiation and the percentage of neutrophils ($p < 0.05$), and also with the percentage of plasma cells ($p < 0.001$), whereas negative correlation was found between the effect of irradiation and the percentage of lymphocytes ($p < 0.05$). (Fig. 2). For mice aged 1.5 months the coefficients of correlation (r) were not significant, except r for eosinophils ($p < 0.05$).

Analysis of the whole population of mice revealed statistically significant positive correlation between the effect of irradiation and fractions of plasma cells ($p < 0.001$), neutrophils ($p < 0.001$), and myelocytes and metamyelocytes ($p < 0.01$), and negative correlation between the effect of irradiation and the relative percentage of lymphocytes ($p < 0.001$).

The results thus showed that with an increase in the relative percentage of neutrophils and their precursors, and also of plasma cells in the spleen, stimulation of luminol-dependent Chl of the cells was observed after laser irradiation. We know that the number of neutrophils and plasma cells in the spleen is increased in chronic infections and certain other diseases [1]; evidently in these cases an increase of sensitivity to laser irradiation must be expected. The results confirm the previous hypothesis for other cell systems [6], that the effect of laser irradiation depends on the physiological state of the irradiated object, and that the response of normally functioning cells and tissues to it is weak.

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KERATIN METABOLISM IN THE EPIDERMIS AND HAIR OF MICE WITH EXPERIMENTAL DIABETES

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KEY WORDS: keratin; metabolism; experimental diabetes

The study of keratinization of the epidermis in diabetes is of great interest, because skin infections and nonspecific pruritis frequently occur in this disease, and the turgor of the skin is reduced. The study of the metabolism of epidermal keratin, a basic protein with a barrier function and also, possibly, facilitating the development of pathological changes in the skin, and also the study of the keratin metabolism of the hair may aid both research in the specific treatment of diabetes and the creation of a noninvasive test for its diagnosis.

In this investigation we studied the rate of biosynthesis and breakdown of prekeratin and keratin with the aid of ^{14}C-glycine. To characterize the process of keratinization in the epidermis, the content of –SH and –S–S– groups in prekeratin also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice in which diabetes was induced by a single intraperitoneal injection of streptozotocin, in a dose of 180 mg/kg body weight. Diabetes developed on the 2nd or 3rd day. The blood glucose level varied between 300 and 450 mg%. The animals were kept in this state for 1 month. The rate of formation and breakdown of keratin and prekeratin was estimated on the basis of incorporation of ^{14}C-glycine into

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