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ACTION OF LASER RADIATION ON PEROXIDE CHEMILUMINESCENCE OF WOUND EXUDATE

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KEY WORDS: laser radiation; chemiluminescence; wound

Low-energy radiation of helium-neon lasers with a wavelength of 632.8 nm has recently begun to be used in experimental and clinical practice. The stimulating action of laser radiation on regenerative processes in various organs and tissues has been demonstrated [1, 4, 5, 14]. Repair processes are accelerated by this factor, the area of the wound surface is quickly reduced, the development of granulation is accelerated, proliferation of connective tissue and epithelial cells is stimulated, phagocytosis is activated, and growth of microorganisms is slowed [3, 7].

We know that the principal role in the development of virtually every pathological process is played by lipid peroxidation (LPO), the state of which affects cellular metabolism, including the proliferative activity of cells [9, 12]. However, there is as yet no sufficiently simple and adequate method of estimating the effect of the LPO level on the rate of wound healing under the influence of laser irradiation. Recording chemiluminescence (CL) in the presence of hydrogen peroxide (H_2O_2), or peroxide CL, is a rapid and convenient method of monitoring the state of LPO and proteins in biological fluids [2, 13].

The aim of this investigation was to develop a method of recording peroxide CL of wound exudate and to study the effect of laser radiation on it.

EXPERIMENTAL METHOD

Experimental investigations of the exudate of wounds involving skin and fascia, healing by second intention, were undertaken.

The wound exudate for chemiluminescence analysis was taken during dressing by means of standard sterile filter paper disks 10 mm in diameter. The discs were applied to the wound surface and the exudate collected for 15 min. The disks were then placed in a flask

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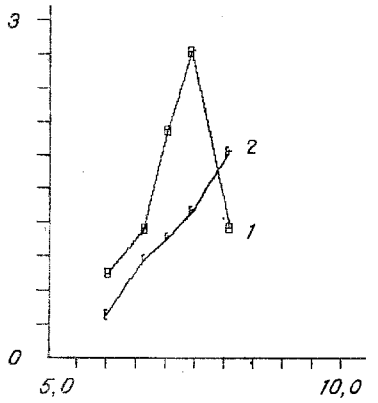

Fig. 1. Dependence of steepness of decay of flash (1) and normalized light sum (2) on pH of solution. Abscissa, pH of solution; ordinate, values of K and S (in rel. units).

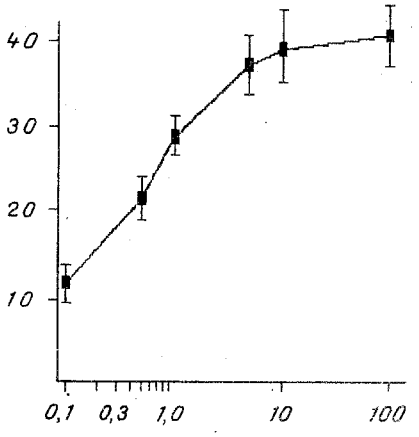

Fig. 2

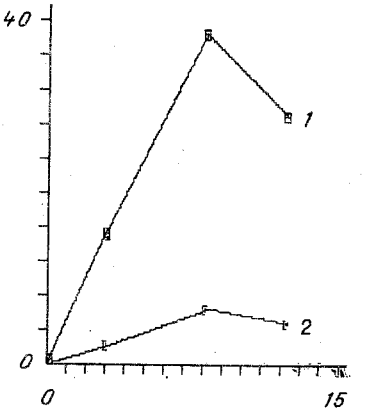

Fig. 3

Fig. 2. Dependence of relative decrease in S on power density of radiation of helium-neon laser during treatment of wounds in children. Abscissa, power density of radiation (in mW/cm^2); ordinate, value of S (in % of initial value).

Fig. 3. Dependence of change in light sum of CL flash of pure catalase, at low levels before (1) and after laser irradiation (2). Abscissa, catalase activity; ordinate, value of S (in rel. units).

TABLE 1. Dependence of Light Sum of CL Flash in Presence of H_2O_2 on Temperature of Solution (M ± m)

Temperature, °C	Test object		
	erythrocytes	serum	exudate
5	31±4	1954±293	1273±140
20	78±8	2940±441	2141±236
40	234±21	5730±764	4841±437

TABLE 2. Effect of Catalase on Light Sum of CL Flash of Tissue Exudate in Presence of H_2O_2 before and after Laser Irradiation (M ± m) in Presence of H_2O_2 before and after Laser Irradiation (M ± m)

Time of determination	Catalase activity, rel. units				
	0	53	110	300	500
Before irradiation	1284±311	10,412±0,01	4,311±0,01	1,096±0,01	0,054±0,01
After irradiation	989±105	9,092±0,01	3,844±0,01	0,516±0,01	0,011±0,01

containing 3 ml of 0.1 M phosphate buffer, pH 7.4, and then eluted for 15-30 min during mechanical shaking. Next, 0.2 ml of the eluate was withdrawn to determine the concentration of apo-B-lipoproteins by turbidimetry. To the residual solution 1 ml of 3% H_2O_2 was added, after which CL was measured for 10 min on a beta-counter, with an additional photoelectronic multiplier (RackBeta, from LKB, Sweden). Addition of H_2O_2 to the solution of tissue exudate was accompanied by the development of a flash of CL, followed by its decay. All the curves obtained were approximated to the exponential equation $I = I_0 + I_1e^{-Kt}$ by means of the Nelder–Mead numerical method on an Apple II computer (USA). The normalized light sum of the CL flash was determined from the ratio between the total light sum and the concentration of apo-B-lipoproteins. Changes in four parameters of CL were taken into consideration: S) the normalized light sum, I_0) steady-state CL, I_1) maximal value of the CL flash, K) a coefficient characterizing the steepness of fall of the exponential curve.

The wounds and specimens were irradiated by the OKG-12 helium-neon laser, emitting monochromatic light with a wavelength of 632.8 nm.

EXPERIMENTAL RESULTS

To find the optimal conditions for measuring CL of the tissue exudate in the presence of H_2O_2, as a first step its dependence on the pH of the solution was studied, for we know that CL depends essentially on pH [6]. It was found that with an increase in pH S increases and extreme values of K are found with a maximum at pH 7.4 (Fig. 1). In all subsequent measurements of CL, the flash was therefore recorded in phosphate buffer with pH of 7.4.

The study of changes in CL depending on temperature showed that with a rise of temperature the value of S also increased (Table 1). All subsequent experiments were accordingly carried out at 20°C.

The study of the action of laser radiation on reparative regeneration of wounds showed that CL of the tissue exudate decreased after irradiation, the state of the wound improved, and the rate of its healing increased. Dependence of the relative reduction in S (as a percentage of its original value) on the power density of radiation of the helium-neon laser during wound treatment is shown in Fig. 2. With low relative power of radiation (from 0.5 to 5 mW/cm^2) sudden quenching of CL was observed. With an increase in the powder density of laser radiation the relative decrease in the normalized light sum of CL flattened out at the steady-state level. The question of the reason for this accordingly arises.

We know that laser irradiation of tissues is accompanied by increased catalase activity [8, 11]. The next step was accordingly to study the effect of radiation of the helium-neon laser on activity of pure catalase. It was found that laser radiation activates catalase sharply, so that the light sum of the CL flash in the presence of H_2O_2 is reduced (Fig. 3). Similar experiments were carried out with the addition of catalase led to a decrease in S of CL, and subsequent laser irradiation caused it to fall even more. The higher the catalase activity the greater the decrease in the intensity of CL.

These results showed that catalase effects both the intensity of CL in the presence of H_2O_2 and processes of wound healing. Catalase decomposes H_2O_2 without forming active forms of oxygen, capable of developing LPO and inducing CL. Thus catalase acts as a water-soluble antioxidant and, when activated by laser irradiation, stimulates reparative regeneration of wounds.

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CHANGES IN COLLAGEN METABOLISM DURING CHRONIC ELECTRICAL STIMULATION OF THE MESENCEPHALIC RETICULAR FORMATION

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The formation of behavioral, emotional, and somato-autonomic reactions in stress takes place with the participation of the brain reticular formation [7]. Stimulation of the mesencephalic reticular formation most frequently causes the blood pressure to rise, due to increases in the heart rate, myocardial contractility, and vascular tone [4]. Electrical stimulation of this region leads to changes in activity of the hypothalamo-hypophyseo-adrenocortical system [8], which may be accompanied by metabolic changes.

The aim of this investigation was to study the character of collagen metabolism in the aortic wall and myocardium during long-term and frequent electrical stimulation of the mesencephalic reticular formation.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 26 adult rabbits weighing 2.5-3 kg. Under local procaine anesthesia bipolar nichrome electrodes were inserted into the experimental and control animals into the reticular nucleus of the tegmentum mesencephali, at coordinates taken from a stereotaxic brain atlas (AP +6, V −15, S −2.5). The animals were used in the experiments 10-11 days after the operation. Square pulses (3-4 V, 70 Hz, 0.5 msec, duration 1 h) were used for electrical stimulation on alternate days for 30 and 90 days. At the

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