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Chemiluminescence response of human neutrophils to He-Ne laser irradiation (*in vivo* and *in vitro*)

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ABSTRACT.

He-Ne laser irradiation ($0.01\text{--}6\text{ J/cm}^2$) of the blood and neutrophile suspension *in vitro* was shown to modulate reactive oxygen species (ROS) production in healthy donors. Intravascular laser irradiation of the blood (5 mW, 30 minutes, daily) of the patients with chronic gastric ulcer during first 5 days resulted in increasing stimulant-induced ROS production in patients with the low initial chemiluminescence response and its decreasing in patients with the high initial chemiluminescence response.

Key words: He-Ne laser, chemiluminescence, reactive oxygen species, neutrophils.

Introduction

The strong and growing interest in studying of mechanisms of the low-energy laser irradiation action on inflammation-related functions of phagocytic cells has been marked during recent years. In part, have shown that N_2 laser ($\lambda = 337.1\text{ nm}$) ⁽¹⁾, He-Ne laser ($\lambda = 532.8\text{ nm}$) ⁽²⁾, pulsed dye laser ($\lambda = 534\text{--}558\text{ nm}$) ⁽¹⁾, Ruby laser ($\lambda = 694.3\text{ nm}$) ⁽³⁾ and infrared GaAlAs laser ($\lambda = 820\text{ nm}$) ⁽⁴⁾ can modulated the phagocytosis of particles and production of

reactive oxygen species (ROS) by leukocytes and splenocytes. On the other hand, during the last decade, numerous reports have outlined the techniques and treatment results in patients with different diseases treated with these lasers (^{5,6}). Earlier, we have found the alteration of monocyte and neutrophils inflammation mediator profile (Tumor Necrosis Factor- α and ROS) in patients with gastric ulcer (^{7,8}). Intravascular irradiation of the blood by the He-Ne laser is successfully used for treatment of this disease (⁹). Here we address a question, whether the in vitro and in vivo He-Ne laser irradiation influences on neutrophil functions (ROS production) in the healthy donors and gastric ulcer patients.

Materials and methods.

A group of sixteen subjects aged from 30 to 45 years has been studied: 10 healthy donors (mans) and 6 patients with chronic gastric ulcer (both sexes). All diagnoses were confirmed gastroscopically. The patients did not receive any drugs before or during the study. The source of light was a He-Ne laser (model LG-92, Russia), 5 mW in output power on the end of the light-conductor. The intravascular laser blood irradiation during daily with the exposure 30 minutes (light-conductor in cubital vein) 5 days were performed (¹⁰). Blood was received by venepuncture performed before and after intravascular irradiation between 09:00 hours and 11:00 hours.

Neutrophils were isolated from heparinized blood (10 U/ml) by centrifugation at 800 g for 30 min on a discontinuous Ficoll/Verografin gradient (1.077 g/ml and 1.119 g/ml) and were suspended in Hanks' balanced salt solution for 30 min at 25°C before being used. There were

about 90% neutrophils in the cell suspension; their viability, assessed by trypan blue exclusion, was more than 96%.

For in vitro irradiation, a blood was diluted by Hanks' balanced salt solution in ratio 1:10. A luminometrical test-tubes with a bottom diameter of 9 mm was filled with 1 ml of dilution blood or cell suspension. Irradiation of samples were performed in measuring chamber of Luminometer 1251 between two mirror semispheres at 37°C. The light-conductor was immersed into a test-tube on 1/2 value of cell suspension and the cells were irradiated 2 sec, 10 sec, 60 sec, 5 min and 20 min (0.01, 0.05, 0.3, 1.5 and 6 J/cm³, respectively) at continuous mixer. As a control, 1 ml aliquots at the same blood or neutrophil suspension were kept under the same conditions but was not irradiated.

The production of reactive oxygen species was determined chemiluminometrically immediately after the irradiation by using an automatic luminometer model 1251 (Wallac, Finland) at 37°C in the presence of luminol (Sigma, USA) as was described previously (⁷). Briefly, 100 µl luminol, to the final concentration of 25 µM, was added to 800 µl dilution blood or neutrophil suspension containing 4x10⁵ cells in a test-tube. After measuring the spontaneous chemiluminescence (CL) (15 min) 100 µl calcium ionophore A23187 (Sigma, USA) (final concentration = 1 µM) or 100 µl activator of protein kinase C - phorbol-12-myristate-13-acetate (PMA) (Sigma, USA) (final concentration = 10 nM) was added to the test-tube and the CL was registered for 60 min.

The data obtained were analysed by using the LUMOGRAF program on an IBM-PC computer (¹¹). To estimate PMA- and A23187- induced CL the following coefficients were calculated:

$$C_{PMA} = \left(\int_0^{60} I_{PMA} - \int_0^{60} I_{SCL} \right) / \int_0^{60} I_{SCL},$$

$$C_{A23187} = \left(\int_0^{60} I_{A23187} - \int_0^{60} I_{SCL} \right) / \int_0^{60} I_{SCL}.$$

where $\int_0^{60} I_{PMA}$, $\int_0^{60} I_{A23187}$ and $\int_0^{60} I_{SCL}$ are integral for 60 min of the chemiluminescence response in presence of PMA (or A23187) and Henks' solution (spontaneous CL), respectively. The effect of laser irradiation was estimated as ratio integral value of spontaneous or induced chemiluminescence to control (nonirradiated) samples CL.

Results.

In the first series of experiments the influence of laser irradiation on the blood CL was studied. It was shown that laser irradiation activate spontaneous CL in the blood of healthy donors (table). This activation influence was more expressive at the first minutes after irradiation, what conform with data, obtained by Karu et al. for He-Ne laser-laser-induced CL response of splenocytes (²).

The final effect of the laser irradiation on PMA-induced CL was depended on the initial values of stimulated CL (C_{PMA}). In fact, we may divided examined sample to three groups, according to the C_{PMA} value: (1) activation of the CL-response by irradiation (dose-dependent effect was noted in the interval from 0.01 to 0.3 J/cm²) (tree subjects, C_{PMA} : 0.11, 0.12, 0.93); (2) the slight modulation of the CL-response by irradiation (dose-dependent effect was not shown) (five subjects, C_{PMA} : 1.1, 1.7, 4.0, 5.4, 7.3, 8.0); (3) irradiation caused decreasing of the CL-response (dose-dependent effect) (two subjects, C_{PMA} : 1.1, 5.4). Thus, activating dose-dependent effect of irradiation was observed

Table. The influence of He-Ne laser irradiation (0.3 J/cm^3) on CL response of human blood in healthy subjects (% control). Each meaning represent of triplicates.

SCL - spontaneous CL. Level of significance: * $p < 0.05$.

Subjects	SCL, integral for		Induced CL	
	15 min	60 min	PMA, 10 nM	A23187, 1 μM
A	127*	103	120*	113*
B	125*	114*	136*	422*
C	81*	88*	101	91*
D	97	98	107	116*
E	110*	111*	139*	96*
F	115*	108	97	112*
G	129*	105	151*	135*
H	133*	122*	91*	109*
I	137	109*	105	111*
J	140*	103	120*	113*

on low initial values of the PMA-induced CL ($C_{\text{PMA}} < 1$).

When A23187-stimulated blood was irradiated, the dose-dependent action on CL was not obvious than in experiments with the PMA-induced blood. The groups structure in this series of experiments was differ from such to the PMA-induced CL.

Laser irradiation, in 50% of oases (irradiation doses were 0.05 and 0.3 J/cm^3) was resulted in decreasing of time of reaching the highest CL value induced oxidative burst (A23187 or PMA) (Fig. 1, for PMA data is not shown). The

kinetic curve of CL-response of neutrophils is dependent on the rate of myeloperoxidase release⁽²⁾ It may be possible, that laser irradiation accelerate neutrophil degranulation process.

In the second series of experiments, we studied the irradiation effect on spontaneous and induced CL of neutrophils suspension. Cells irradiation (doses were 0.3 - 0.6 J/cm³) dose-dependently activated spontaneous CL, and, practically, had no influence on PMA-induced CL ($p > 0.05$) (Fig. 2).

To research the intravascular blood irradiation influence on neutrophils ROS production, we investigated "immediate" (measures of induced CL level was carried out at 15 min after irradiation) and "distant" (twenty-four hours after irradiation) effects. We found out, that intravascular blood irradiation forced undulating alterations of PMA- or A23187- induced CL with gradual decreasing of fluctuations during 5 days of laserotherapy. Intravascular irradiation of the blood by He-Ne laser on the next day after the first treatment resulted in increasing A23187-stimulated and PMA-induced CL in patients with the low initial CL-response

Fig. 1. The effect of He-Ne laser irradiation on the A23187-induced blood CL in healthy subject ($A23187 = 1 \mu M$). SCL - spontaneous CL.

Fig. 2. The effect of He-Ne laser irradiation on neutrophils CL. (1) spontaneous CL; (2) PMA-induced CL ($PMA = 10nM$). Each columns represent means of triple measuring of six healthy subjects. % control. Level of significance: * $p < 0.05$.

and its decreasing in patients with the high initial CL-response. The average minimum value of A23187- and PMA-induced CL was observed on third day (after two irradiation treatment) (Fig. 3). (for PMA-induced CL data is not shown). Comparisons of experiments in vivo and in vitro revealed that modulating "immediate" influence of the blood irradiation had similar results.

As we know, kinetics of luminol-dependent chemiluminescence of neutrophils is determined by proceeding activity of following processes: neutrophils degranulation with myeloperoxidase release, luminol diffusion into the cells and its interaction with intracellular-production ROS, activity of enzymes of formation and metabolism of reactionable elements, which interacted with luminol: membranoconnected NADPH_2 -oxidase, superoxide dismutase, catalase or myeloperoxidase (¹²). Really, NADPH_2 -oxidase is a complicated multicomponent ferment complex and its activation can be proceeding similar with mechanism, proposed to explanation of activation effect of laser beam on flavin components of the respiratory chain (¹³). Activation influence of He-Ne laser on catalase and superoxide dismutase was demonstrated too (^{14,15}). Although, in other works that fact was not confirm (¹⁶). On the other hand, the most of this processes is directly connected with cellular membrane or with neutrophil intracellular lipids. Therefore, it is possible, that in the base of biostimulating influence of laser irradiation on neutrophils may be structure-functional recombinations of membrane elements with regulating orientation.

Thus, in our report was demonstrated, that laser irradiation (He-Ne laser, $\lambda = 632.8 \text{ nm}$) causes activation of

Fig. 3. The effect of intravascular He-Ne laser irradiation on the A23187-induced blood CL in six patients with gastric ulcer (A23187 = 1 μ M). Each point represent of triplicates.

spontaneous ROS production by human neutrophils under irradiation, both in the blood and in the cells suspension. Based on our data, we may propose that the results of the blood irradiation, in vivo and in vitro, are dependent on initial values of induced CL, irradiation dose and, possible, on modulation influence of other blood cells.

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