

Action of pulsed visible and near IR laser radiation on oxidative metabolism of cells evaluated by chemiluminescence measurement

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ABSTRACT

Luminol-amplified chemiluminescence of was recorded after irradiation with laser radiation at 632.8 and 820 nm. Following cellular systems were used as objects of irradiation: blood of healthy donors, blood of patients with colon cancer or acute respiratory illness, blast cells of patients with acute leukemia. The irradiation was suppressing the oxidative metabolism of cellular systems under study.

1.INTRODUCTION

One of the methods used to evaluate changes in the oxidative metabolism of cells and tissues is the measurement of chemiluminescence (CL) associated with the generation of active oxygen forms (AOF)¹. Intact phagocytizing and nonphagocytizing cells have been known to feature spontaneous chemiluminescence (SCL) reflecting the original status of metabolic processes.^{1,2} When a cell is subjected to various stimuli, its chemiluminescence response may change, i.e., the amount of AOF generated by the cell may increase or decrease.²

2.LASER RADIATION AT 820 nm HAS NO STATISTICALLY SIGNIFICANT EFFECT ON CHEMILUMINESCENCE OF HEALTHY HUMAN BLOOD

Used in this work was whole blood randomly sampled from clinically healthy persons (6 men and 22 women 30-50 years old) subjected to yearly prophylactic examination. Blood samples were taken from the cubital vein at 9-10 a.m. 200 μ l of heparinized blood was diluted in the proportion one part blood to ten parts Medium 199. The blood samples taken from each donor were tested for SCL and CL induced by adding *Candida albicans* as an object of phagocytosis prior to irradiation and for CL after the irradiation. For irradiation, 100- μ l samples of diluted blood were placed in 5-mm-dia. wells in 96-well plates and irradiated from above with a Model Biotherapy 3ML semiconductor laser (Omega Ltd., Great Britain) at λ =820 nm. The irradiation dose amounted to 1×10^4 J/m², the pulse repetition frequency was 292 Hz, and the exposure time, 13 seconds. Irradiation was carried out in a dark room.

To measure CL, the irradiated and control samples were transferred to special plastic test tubes containing each 100 μ l of Medium 199 and 100 μ l of a luminol solution (Serva) with pH 7.2. When measuring the phagocytosis-induced CL, a suspension of *Candida albicans* killed preliminarily by heating and opsonized with cattle serum was added in a concentration of 10^8 particles per milliliter to the test tubes instead of Medium 199. Kinetic CL curves were recorded for 2 hours with a Model CL-3603 chemiluminometer (Dialog, Moscow, Russian Federation), the data being automatically entered into an IBM PC/AT personal computer. The leukocyte count and blood-picture were determined for each sample.

The significance of differences was determined by means of the χ -square test for the alternative distribution and the u-test (Wilcoxon-Mann-Whitney). The graphical and statistical processing of the data was carried out by means of the commercially available program packages QPRO and STATGRAPHIC.

The results of measuring the CL of blood samples from 28 donors showed that the kinetic SCL curves differed in shape so that could be classified under three main types, and so the donors could accordingly be subdivided into three groups. The first type of curves is characterized by the presence of a single maximum in the interval 40-60 min after the start of recording (curve 1 in Fig. 1a). The second type reflects a slow increase of CL, its maximum falling within the interval 90-110 min after the start of recording (curve 1 in Fig. 1b). The third type has a double-wave character (curve 1 in Fig. 1c). The first type was observed in 4 donors, and the second and third ones, in 12 persons each.

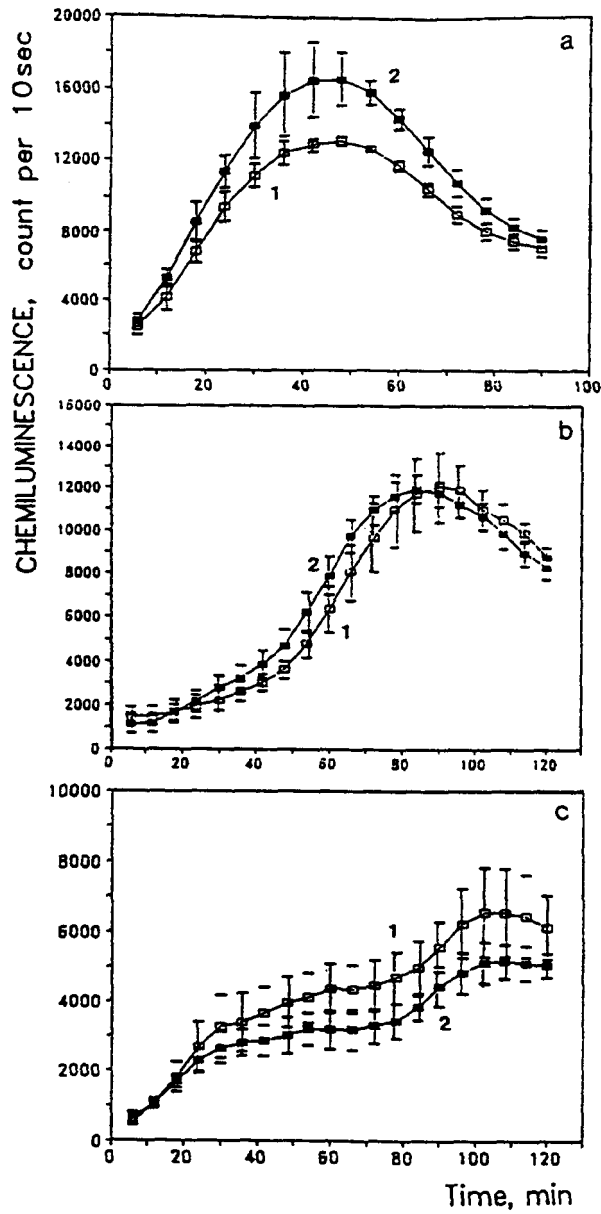


Fig. 1. Kinetic CL curves after exposing three groups (a,b,c) of clinically healthy blood samples to laser light (820 nm, 292 Hz, 1 J/cm²).

The situation with irradiated blood samples proved to be following. The significance of the differences in CL between the irradiated and control blood samples was estimated by means of the u-test (Wilcoxon-Mann-Whitney) separately for the time intervals 40-60 min (here-inafter referred to as the first point, see Fig. 1) and 90-110 min (second point, see Fig 1). It was only in a single case that a significant effect of irradiation, namely, a 28% stimulation at the first point ($p < 0.01$, Fig. 1a), was observed in the first group of donors, the differences between the irradiated and control blood samples in the rest of the cases being insignificant. In the second group of donors, significant changes in CL as a result of

irradiation were observed in two cases, CL being stimulated by 20-30% ($p < 0.01$ and $p < 0.05$ respectively) at the first point of the CL curve (see, for example, Fig. Ib). In the third group of donors, there occurred in five cases the inhibition of the CL blood as a result of irradiation: CL rose by 15-40% ($p < 0.05$) at the first point of the CL curve in three cases and by 17-22% ($p < 0.05$) at the second point in two cases (see, for example Fig. Ic). Thus, statistically significant effect of laser radiation on the CL of blood was observed in 8 cases out of 28, CL being stimulated in three cases and inhibited in five cases (Table 1). These data do not allow one to speak of any marked effect of laser radiation at $\lambda=820$ nm on the oxidative metabolism in blood cells, although the third differs significantly from the first two ($\chi^2 = 5.52$, $p < 0.05$). Attention is drawn to the existence of some relationship between the irradiation effect and the initial shape of the SCL curves. To illustrate, in the third group which is characterized by CL curves with two maxima and relatively low SCL values (curve 2 in Fig. Ic), the only effect (if any) was inhibitive (see Table 1). At the same time, the effect of laser radiation (if any) in the first and second groups was stimulative only (see Table 1). We conducted no special investigation with a view to revealing the cause of the difference in shape between the CL (curves 1 in Fig. 1).

TABLE 1
Number of cases of significant differences in the magnitude of CL
between irradiated and control blood samples (by the x-test)
Irradiation effect

Group	No. of donors	Significant differences		
		Stimulation	Inhibition	Insignificant differences
I	4	1 ($p < 0.01$)	0	8
n	12	2 ($p < 0.05$)	0	10
m	12	0 ($p < 0.01$)	5 ($p < 0.05$)	7

The results of this work support the results obtained earlier for other cellular systems³ to effect that the action of laser light depends on the physiological condition of the exposed object, normally functioning cells and tissues being practically inresponsive to it. This result may be of importance from the standpoint of the development of methods for laser therapy and the evaluation of its effectiveness.

3.LASER RADIATION AT 820 nm SUPPRESSES CHEMIUMINESCENCE OF BLOOD OF PATIENTS WITH ACUTE RESPIRATORY ILLNESS

The peripheral blood of a healthy female donor (26 years old) was studied during two periods (March and September) of acute viral respiratory illness with clinical manifestation of common cold (rhinorrhoea, sneezing, nasal congestion with a syndrome of phagotonsillitis without systemic syndrome) and a *Herpes simplex* lesion on the upper lip.

In both periods the clinical features as well as the duration of the illness were practically the same. The acute period of the illness lasted 3 days, and a convalescent period of 6-7 days. The illness resolved without sequelae. No medical treatment was carried out. The measurements of blood chemiluminescence were performed every third day during 28 days from the beginning of the illness. The blood was collected by venipuncture of a finger stick always at the same time of day near 12 noon to avoid circadian fluctuations in the activity of phagocytes.⁴

Fresh peripheral blood (300 μ l) was collected in a heparinized test-tube and diluted ten times with Medium 199. The number of leucocytes was counted and smears for determination of cellular composition were prepared in every experiment. The smears were fixed with methyl alcohol and stained with Giemsa. Two hundred cells were identified in every smear. The irradiation parameters and procedure as well as CL measurements technique were the same as described in Section 2.

Figure 2 presents typical examples of kinetic curves of spontaneous luminol-amplified chemiluminescence (SCL) peripheral blood of the donor during the period of the illness (curve 2) and normal conditions of health (curve 3). The blood in the acute period of the illness (the first 3 days) is characterized by a rapidly increasing SCL during the first 40 min of the recording followed by a decrease in SCL (curve 1). The SCL of normal blood increases very slowly during the first 60 min of recording (curve 3). The convalescent period (approximately from 4th to 9th days of the illness) is characterized by

gradual changes in shape of CL curve from the first type (curve 1) to the third one (curve 3), as illustrated by curve 2 (the 6th day of the illness).

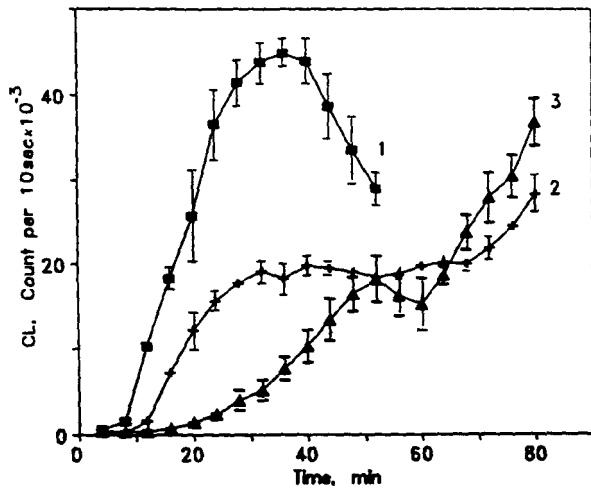


Figure 2. Typical kinetic curves of spontaneous chemiluminescence of blood during periods of (1) acute illness (2nd day), (2) recovery (6th day) and (3) in healthy condition (13th day)

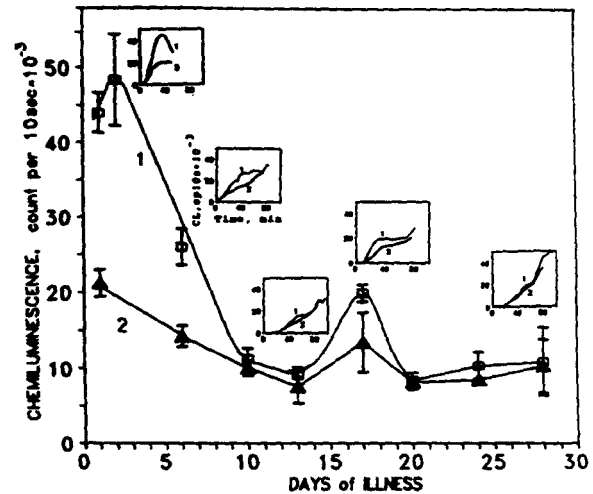


Figure 3. Values of (1) SCL and (2) CL of irradiated (820 nm, 292 Hz, $1 \times 10^4 \text{ J/m}^2$) blood at the time points 40 min after the beginning of CL recording during whole periods of measurements. The insets illustrate respective CL curves of (1) SCL and (2) CL of the irradiated blood

Figure 3 (curve 1) does not show the whole kinetic curves of SCL (some examples are shown in the inserts) but only one point in it for every day (40 min after the beginning of CL recording). As seen in Figure 3 (curve 1), the SCL at this particular time point is high during the acute period of the illness (the first 3 days), then decreases during the recovery period (approximately 6 days after the acute period) and is at a normal level in 10th day after the beginning of the acute period. The value of the SCL near 1×10^4 counts per 10 s is a normal value for this particular donor in our experimental conditions. The changes in SCL were practically the same (within the limits of experimental error) during both periods of the illness (March and September).

The CL of the whole blood can, in principle, originate from both plasma and cellular components. We performed special measurements of the CL of the blood plasma of the donor. In our experimental conditions and measurements technique the luminol-amplified SCL of plasma was not above the background counting neither during the period of the illness nor in healthy conditions (results not shown). This result clearly evidences that the CL recorded in our experiments originates only from the cellular components of blood.

The question of interest is from which types of cells CL originates. In our earlier experiments we established correlation between the effect of laser irradiation (percentage changes of CL when murine splenocytes were irradiated at 820 nm and 292 Hz) and percentage of various cells in the irradiated suspension. In the present work the same approach was used. The results of linear regression analysis are presented in Table 2 and 3. Table 2 contains correlation coefficients between SCL and percentage of various cells in blood, and Table 3 shows correlation coefficient between the effect of irradiation (suppression of SCL when irradiated at 820 nm, 292 Hz, $1 \times 10^4 \text{ J/m}^2$) and percentage of various cells. The distribution of data has been found to be normal.

It is noteworthy that SCL depended practically on the ratio of all cells measured: On the percentage of lymphocytes and neutrophils statistically significant; and the correlations with percentage of monocytes and eosinophils were very close to the statistically significant values (Table 2). The results of the correlation analysis between the effect of irradiation and percentage of various cells in blood were quite different from the correlations with SCL (Table 3). The only statistically significant correlation has been found with the ratio of eosinophils. Other correlation coefficients were far from statistical significance. One additional remark should be made. The present study was concerned with the description of a

phenomenon, a possibility of suppressing oxidative free radical processes in human blood and the medical aspects of the problem were not under study.

As a result of present experiments, we found a possibility to suppress free radical processes in human blood: maximally 60% of the CL was suppressed in certain cases (as a dependence of health conditions of the donor).

Table 2. Correlation coefficient r between the spontaneous chemiluminescence (SCL) and percentage of various cells in the blood or murine spleen suspension

Cells	r	
	Human blood $n = 10$	Murine spleen ⁵ $n = 13$
Lymphocytes	-0.644*	-0.015
Monocytes (macrophages)	0.452	0.153
Neutrophils	0.587+	0.095
Eosinophils	0.540	0.004

Data from reference 11.
n = number of correlation pairs.
* $p < 0.05$; † $p < 0.08$.

Table 3. Correlation coefficient r between the effect of irradiation (suppression of CL when irradiated at 820 nm, 292 Hz, 1×10^4 J/m²) and percentage of various cells in the blood or murine spleen suspension

Cells	r	
	Human blood $n = 10$	Murine spleen ⁵ $n = 13$
Lymphocytes	0.099 -	-0.417 -
Monocytes (macrophages)	0.221	0.031
Neutrophils	0.204 -	0.3411
Eosinophils	0.594*	0.6681

Data from reference 11.
n = number of correlation pairs.
* $p < 0.08$; † $p < 0.05$.

4. LASER RADIATION at 820 nm SUPPRESSES THE CHEMILUMINESCENCE OF BLAST CELLS IN PATIENTS WITH HEMOBLASTOSES

Used in the work have been bone marrow samples taken prior to the start of therapy from 5 patients suffering from hemoblastosis, 3 of them with acute lymphoblastic leukemia (ALL) and 2 with acute myeloblastic leukemia (AMI). Blast cells have been separated in Ficoll-Paque ("Pharmacia", Sweden) gradient ($=1.066$). After centrifuging the whole bone marrow samples, interphase ring cells have been collected, rinsed twice with cold phosphate-buffered saline (PBS), and resuspended in the Medium RPMI 1640 (Flow Lab., U.K.1); the final cell concentration in the medium has been 4×10^8 cells/ml. A smear has then been prepared from each suspension sample thus obtained to determine the cellular composition of the separated fraction and check the quality of the cells. The smears have been fixed with methyl alcohol and Romanovskiy-Giemsa stained. Blast cells in the suspension samples obtained have accounted for over 90% of the total cell content, there being also some admixture of neutrophils, myelocytes, lymphocytes, and eosinophils.

All the patients examined have been treated at the Russian Cancer Research Center's clinic. The chemotherapy scheme for the ALL patients has included vincristine, L-asparaginase, prednisolone, and anthracyclic antibiotics; that for the AML patients, cytosar and anthracyclic antibiotics.

Subject to measurement in each bone marrow suspension sample have been spontaneous chemiluminescence (SCL) and also chemiluminescence (CL) upon addition of a phagocytosis object- *Candida albicans* - and chemotherapeutics and exposure to a semiconductor laser. To measure the effect of the chemotherapeutics, 100- μ l samples of the cellular suspension under test have been placed in the plastic test tubes of a Biolumat Model LB 9500 chemiluminometer (Berthold Co., Wilbad, FRG), each sample being mixed with 6 μ l of a solution of one of the following therapeutics: vinblastine (VB), vincristine (VC), rubomycin (RM), adriamycin (AM), and cytosar (Cy). The final concentration of \ the therapeutics has counted to 1×10^{-6} M. After that, 100 μ l of a 20-mM solution of luminol buffered with a sodium phosphate solution (pH = 7.2) has been added to each test tube, together with 100 μ l of the Medium 199 (Institute of Poliomyelitis and Viruses, Moscow). To study the effect of laser radiation on the CL of blast cells, 100- μ l cellular suspension samples have been placed in the wells of 96-well plates .and then irradiated with a 820-nm semiconductor laser (Model Biotherapy 2ML, Omega Ltd., U. K.1) as described in Section 2. The irradiation parameters and chemiluminescence measurements are also described in Section 2.

It has been found as a result of measuring the SCL of blast cells in 5 patients that their kinetic SCL curves differ in shape, there being two main types of curves, and so the patients can be subdivided into two groups. In patients

of the first group the SCL of blast cells reaches its maximum within 15-20 min after the start of measurements and then decreases. The numerical SCL values in this group are rather high, their maximum being at $(1.2 \text{ to } 1.6) \times 10^3$ counts/10 s. Some examples of such curves are presented in Figs. 4a and 5a (curves 1). In the second group of patients, SCL grows gradually and features no distinct maximum. The numerical SCL values are very low, not over 500 counts/10 s. Examples of such kinetic SCL curves are presented in Figs. 4b and 5b (curves 1).

Figures 4a and b show also the kinetic CL curves of blast cells irradiated with a semiconductor laser at $\lambda=820 \text{ nm}$ (curves 2) the curves of Fig. 4a referring to the patients in the first group and those of Fig. 4b, to the patients in the second group. The control in these experiments have been the kinetic SCL curves of Figs. 4a and b (curves 2). It has turned out that irradiation of the cells inhibits CL in the first group of patients (Fig. 4a) and stimulates it in the second group (Fig. 4b). Thus, irradiation has induced qualitatively different reactions in the cells in the first and second groups of patients.

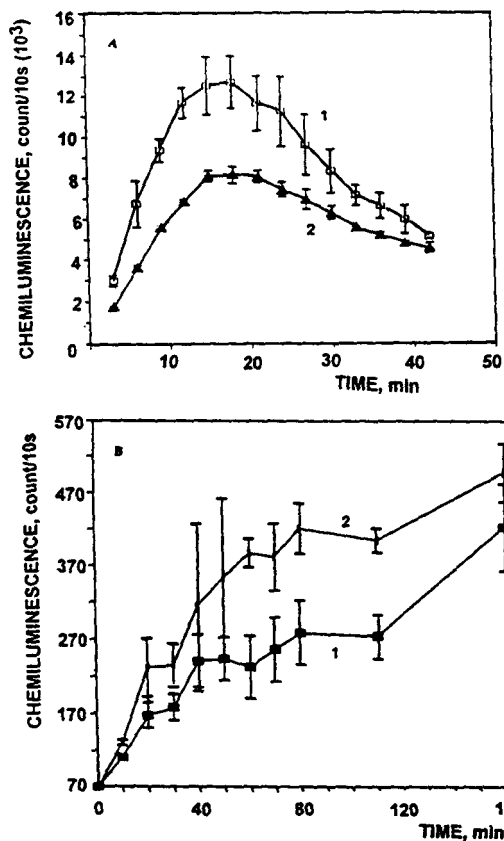


Fig. 4. Effect of laser radiation (820 nm, 292 Hz, $1 \times 10^4 \text{ J/m}^2$) on CL of blast cells in patients of (a) the first group and (b) the second group: 1-SCL; 2-CL after exposure to the laser radiation.

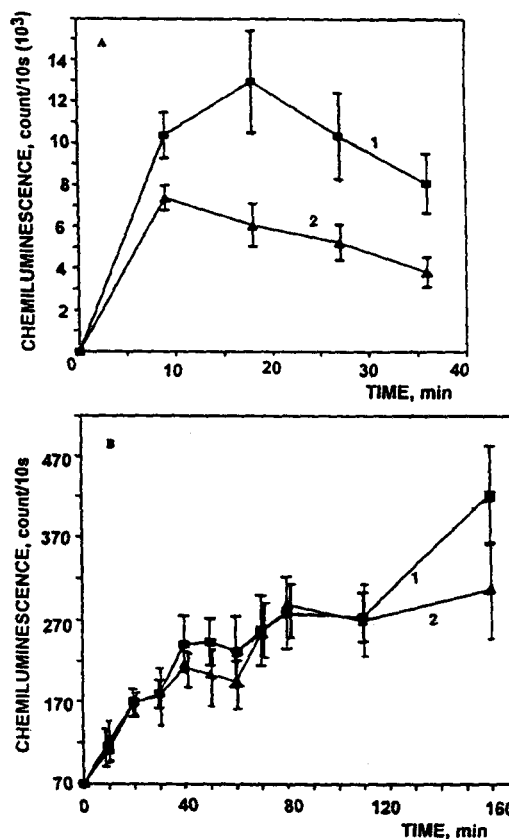


Fig. 5. Effect of VC on the CL of blast cells in patients (a) the first group and (b) the second group: 1- SCL; 2-CL after addition of VC ($1 \times 10^{-6} \text{ M}$).

Figure 5 presents the kinetic CL curves of blast cells treated with one of the chemotherapeutics being tested namely vincristine (VC). It can be seen that VC inhibits CL of the cells in the first group of patients (Fig. 2\3a) and has no effect on that of the cells in the second group (Fig. 2b). Vinblastine (VB) has had a similar effect (inhibition of CL in the first group of patients and no effect in the second group) (Table 4). The rest of the chemotherapeutics studied have had no effect on the CL of blast cells in either group of patients (see Table 4).

A retrospective analysis of the results of treatment of the patients and comparison between the clinical data and the results of the *in vitro* chemiluminescence test (see Table 4) have allowed us to draw the following conclusions. The chemotherapeutics VC and VB and also laser irradiation have inhibited the CL of blast cells in the first group of patients (patients numbered 1,2, and 3 in Table 4). At the same time, the use of VC in the treatment scheme has caused a persistent remission (patients No. 1 and 3). Where use was made of AD and Cy that have had no effect in the chemiluminescence test to treat a patient in the same group (patient No. 2), the treatment effect has been nil. Neither of the chemotherapeutics tested has had any effect on the CL of blast cells in the second group of patients.

Table 4 Effect, of chemotherapeutical and laser radiation on the chemiluminescence of blast cells *in vitro* and treatment results

No.	Patient	Diagnosis	Curve types	Chemiluminescence test						Clinical data	
				Effect on CL						Drug used	Treatment effect
				Laser	VB	VC	AD	RM	CY		
1.	G.	ALL	I	Inh.	Inh.	Inh.	N/E	N/E	**	VD, RM, Cy, P	CR
2.	K.	AML	I	Inh.	Inh.	Inh.	N/E	N/E	N/E	Cy, AM	N/E
3.	L.	ALL	I	Inh.	Inh.	Inh.	N/E	N/E	**	VC, RM, Cy, P	CR
4.	T.	AML	II	Stim.	**	N/E	N/E	**	N/E	Cy, AM	N/E
5.	M.	AML	II	Stim.	N/E	N/E	**	N/E	N/E	VC, RM, Cy, P	PR

Legend:

AD- adriomycin	P- prednisolone
ALL- acute lymphoblastic leukemia	RM- ribomycin
AML- acute myeloblastic leukemia	PR- partial remission
Cy- cytosar	VC- vincristine
CR- complete remission	VB- vinblastine
N/E- no effect	** - no test made

The results obtained are inadequate to make the final conclusion as to the expediency of using the chemiluminescence test to evaluate the individual sensitivity of leukemic cells in human beings to VB and VC. Nevertheless, the like effects of VB, VC, and laser radiation (inhibition of the SCL of blast cells) may form a basis for the future development of a noninvasive chemiluminescence test for the medication sensitivity of cells in patients suffering from hemoblastoses.

5.PULSED LASER RADIATION at 632.8 nm SUPPRESSES THE CHEMILUMINESCENCE OF BLOOD OF PATIENTS WITH COLON CANCER

Subject to investigation was a group of six patients (5 women and 1 men, 45-67 years of age) with cancer of the colon (stages IIIA-IV B) admitted to the clinic of the Russian Cancer Research Center of the Russian Academy of Medical Sciences. Blood samples were taken from the cubital vein at 9-10. .m. 200µl of heparinized blood was diluted in the proportion are pan blood to ten parts Medium 199. The blood samples taken from each donor were tested for SCL and CL induced by adding *Candida albicans* as an object of phagocytes prior to irradiation and for CL after irradiation. The blood of the control group (6 clinically healthy persons) was treated in the same way. The leukocyte count and blood-picture were determined for each sample.

The radiation source used was He-Ne laser with an acoustooptical light modulator and a frequency synthesizer (Model Luch-1, Special Design Department of the Institute of Radio Engineering and Electronics, Russian Academy of Sciences, Fryazino, Russia). The irradiation mode was either continuous-wave (CW) or pulsed, with a pulse repetition rate from 1 to 100 Hz and duty cycle of 50 or 94%.

Blood samples were placed in special round glass cells 110 μl in volume and irradiated from above. The exposed surface area was equal to 0.28 cm^2 , the exposure time amounted to 28 s (CW mode), 30 s (pulsed mode with a duty factor of 94%), or 56 s (pulsed mode with a duty cycle of 50%), and the exposure dose was $5 \times 10^3 \text{ J/m}^2$. Irradiation as well as chemiluminescence measurements were performed in a dark room at room temperature as described in Section 2. The CL kinetics was recorded during 120 min. The graphical and statistical processing of the data obtained were carried out by means of the QPRO and Statgraphics commercial program packages.

The analysis of the blood SCL curves for healthy donors showed these curves to have a bimodal character: The first maximum was observed within 25 -30 minutes of the start of CL recording, and the second, more pronounced maximum occurred within 90-120 minutes. It should be noted that the ratio between the second and the first peak was always in the range 2-2.5. An example of these curves is presented in Figure 6 (curve 1).

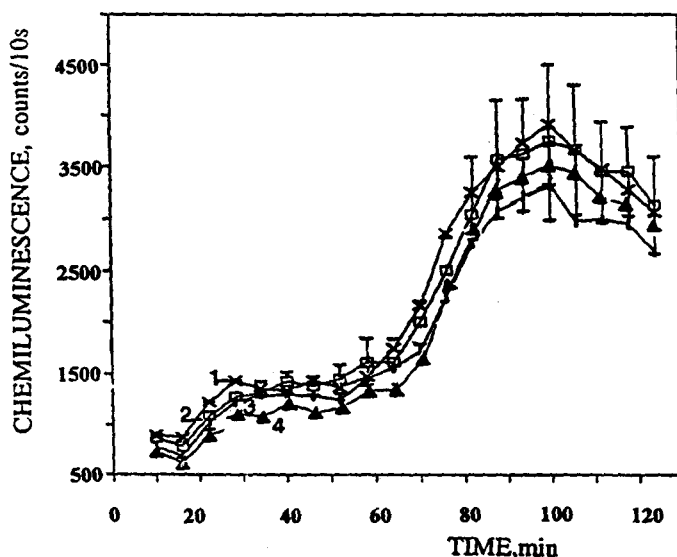


Fig.6. Examples of luminol-dependent blood chemiluminescence kinetic curves for healthy donors: (1) spontaneous chemiluminescence; (2) chemiluminescence following irradiation with CW (632.8 nm) laser light; (3) and (4) chemiluminescence following irradiation with pulsed laser light ($\lambda = 632.8 \text{ nm}$) at a pulse repetition frequency of 1 Hz and a duty cycle of 50% and 94%, respectively.

The character of the blood SCL curves for patients with cancer of the colon (CC) differed from that of the control curves: the first peak (25-30 min after the start of CL recording) rose greatly (5-10 times). A typical example of such curves is presented in Figure 7 (curve 1). The ratio between the second and the first peak was around 0.5. Thus, the blood SCL curves for the CC patients differed from the corresponding curves for the healthy donors both qualitatively and quantitatively. Based on the literature data⁶ it may be assumed that the first peak is associated with the activity of neutrophils and the second, with that of macrophages. There being no sharp increase in the number of neutrophils in the blood samples from the CC patients in comparison with that in the controls, the increase of SCL may apparently be interpreted as the result sensitization with respect to tumor cells. This hypothesis is supported by the fact that SCL per neutrophil in the patients with cancer of the colon was greater than in the healthy donors. Similar results were also obtained when the blood of patients with tumors of other locations was analyzed^{6,7}.

Quantitatively to evaluate the effect of irradiation, we next compared between the percent change of CL compared to SCL at the maximum of the kinetic curves (25-30 min after the start of measurement). Our analysis of the CL curves for the irradiated blood samples allowed us to conclude that CW He-Ne laser radiation under our experimental conditions had no

statistically significant effect on the CL of blood in both the healthy donors and CC patients (curves 2 in Figs. 6 and 7 as typical examples); the effect amounted to $98.3 \pm 4.8\%$, and $100.4 \pm 4.8\%$, respectively.

In contrast to irradiation with CW light, exposing the blood samples from the CC patients to pulsed laser radiation materially inhibited their SCL. The effect depended on the pulse repetition frequency and duty cycle. Figure 7 presents a few examples (curves 3 and 4). At the same time, the effect of pulsed radiation on the CL of blood in healthy donors proved statistically insignificant in the overwhelming majority of cases. Some examples are presented in Fig. 6 (curves 3 and 4). The reason for the increased sensitivity to pulsed laser light of blood in the patients with cancer of the colon in comparison with that in the healthy donors is yet to be explained. The discussion of this problem is beyond the scope of the present paper.

Figure 8 shows the effect of inhibition of the SCL of whole blood as a function of the pulse repetition frequency. As can be seen, the effect on the blood samples from the CC patients (curves 3 and 4) depends, in contrast to those from the healthy donors, both on the pulse repetition frequency (the reduction of the effect with the increasing pulse repetition frequency) and the duty cycle. Radiation with the duty cycle of 50% inhibits SCL more effectively, especially at pulse

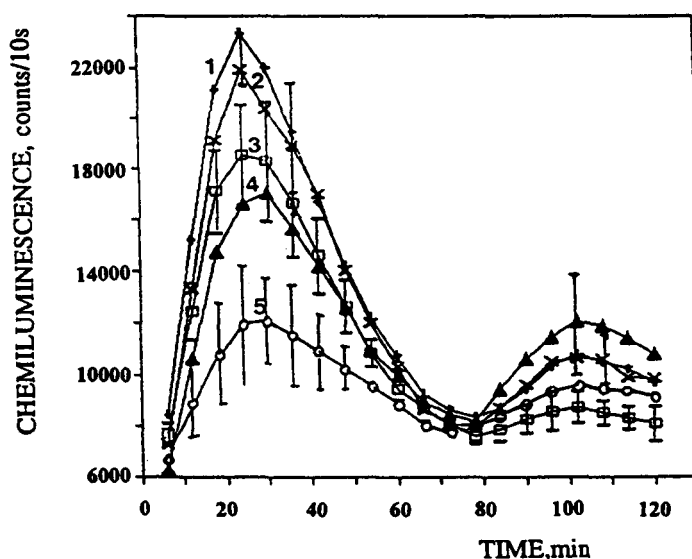


Fig.7. Examples of luminol-dependent blood chemiluminescence kinetic curves for patients with cancer of the colon: (1) SCL, (2) CL following irradiation with 632.8 nm CW laser light; (3) and (4) CL following irradiation with pulsed 632.8 nm laser light at a pulse repetition frequency of 10 Hz and a duty cycle of 94% and 50%, respectively; (5) CL following irradiation with pulsed 632.8 nm laser light at a pulse repetition frequency of 1 Hz and a duty factor of 50%.

repetition frequencies of 1-10 Hz. To illustrate, at pulse repetition frequency of 1 Hz and duty cycle of 50%, SCL is inhibited by 45% (curve 4 in Fig. 8). As the pulse repetition frequency is raised, the effect decreases (Fig. 9). One can delimit the region of effective action of pulsed laser radiation at $\lambda = 632.8$ nm on the SCL of the blood in the CC patients under our experimental conditions: pulse repetition frequency 1-10 Hz at a duty cycle of 50% or pulse repetition frequency of 1 Hz at a duty cycle of 94%.

Thus, the results of this investigation based on the evaluation of the ability of whole blood cells to generate highly toxic oxygen radicals show, first, that they are much more sensitive to pulsed than CW laser radiation (the radiation wavelength and dose being the same). Secondly, the sensitivity of blood in the patients suffering from cancer of the colon to pulsed laser radiation is enhanced in comparison with that of blood in the healthy donors.

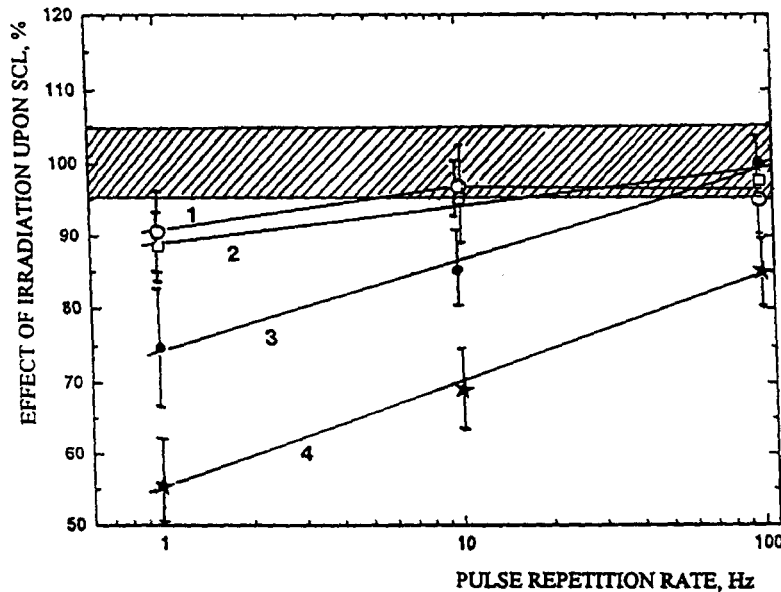


Fig.8. Irradiation effect (inhibition of SCL) as a function of pulse repetition frequency: effect of pulsed 632.8 nm radiation with a duty cycle of 94% on blood from (1) healthy donors and (3) CC patients, and effect of pulsed laser radiation *with* a duty factor of 50% on blood from (2) healthy donors and (4) CC patients. The CL of blood in both healthy donors ($98.3 \pm 3.5\%$) and CC patients ($100.4 \pm 4.2\%$) following the exposure to CW 632.8 nm laser light practically does not differ from SCL ($100 \pm 5\%$) and is indicated by the shaded area.

6. CONCLUSIONS

The present studies were concerned with the description of action of laser radiation on oxidative metabolism (evaluated by luminol-amplified chemiluminescence test) of human blood. It was found that radiation with certain parameters (pulsed light at 632.8 and 820 nm) can suppress the free radical processes in blood of some patients but not that of healthy persons. However, one should emphasize that the medical aspects of the problem were not under study here. The blood of patients was considered as a model system.

7. ACKNOWLEDGEMENTS

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