Changes in Chemiluminescence of Undiluted Blood in Patients with Ischemic Heart Disease during Laser Therapy

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Three sessions of intravenous He-Ne laser therapy markedly decreased the intensity of luminol-dependent chemiluminescence of undiluted blood in most patients with ischemic heart disease. After seven sessions, the chemiluminescence parameters in most patients were similar to those in healthy donors. However, in some patients a 10-session course of laser therapy increased the intensity of luminol-dependent chemiluminescence. A positive correlation was established between the parameters of chemiluminescence of undiluted blood and its viscosity as well as activity of plasma neutrophils determined by the Nitro Blue Tetrazolium test.

Key Words: *undiluted blood; luminol; chemiluminescence; ischemic heart disease; laser therapy*

Neutrophils are known to produce reactive oxygen species responsible for tissue damage during myocardial ischemia [7]. Generation of these species by neutrophils is accompanied by chemiluminescence (CL) [2], therefore determination of CL in suspension of neutrophils was proposed as a method to evaluate the degree of ischemia in the myocardium [12].

Neutrophils are usually isolated from the blood by the density gradient centrifugation [5]. However, this procedure can modify the properties of isolated neutrophils. Some authors propose to measure the neutrophil-dependent CL in undiluted blood, but high optical density of the blood is a serious obstacle in measuring CL, so the blood is always diluted [11], which may affect neutrophils. From this viewpoint, undiluted blood is the best for analysis of neutrophil properties. The possibilities to measure CL in undiluted blood glimpsed in the early 1930s, when mitogenetic radiation of the blood (extremely weak ultraviolet radiation) was recorded [6,8]. Our work shows that in the presence of the amplifier luminol, CL in undiluted blood depends on the donor's health, and its analysis can be used to control the treatment of ischemic heart disease during intravenous laser therapy.

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MATERIALS AND METHODS

The patients with chronic ischemic heart disease were hospitalized and treated with a daily intravenous radiation from an ALOK-1 He-Ne laser (λ=633 nm, beam power at the light guide tip 1 mW). The light guide was inserted into the vein using a conductor needle. Duration of each treatment session was 30 min. Laser therapy was combined with conventional medicamentous treatment. The blood was taken immediately after admission of the patients to the hospital and 24 h after 3, 7 (or 8), and 10 sessions as well as prior to every new session. The blood was drawn from ulnar vein, stabilized with heparin (100-200 U/ml), and processed no later than 2 h after the sample was drawn.

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Chemiluminescence of blood samples (0.2 ml) was recorded at 19-21°C in the single photon counting mode in a Mark-II (Nuclear-Chicago) liquid scintillation counter using 1 ml polyethylene test tubes (Eppendorf), and an EMI 9750QB/1 photomultiplier tube. Blood was transferred into the test tube, and radiation was recorded for 2-3 min. Luminol (Sigma) was then added from the solution of analytical grade dimethyl sulfoxide to a final concentration of 10^{-4} M. After the addition of luminol, CL was recorded for at least 3 min.

The total blood count of all samples was obtained with the help of a Cell-Dyn 2000/1500 automatic analyzer (Sequoia-Turner Corp.). The ability of neutrophils to reduce Nitro Blue Tetrazolium (NBT) was assessed by conventional technique [10]. In addition, the relative activity of neutrophils was evaluated using the cytochemical activity index (CAI), taking into account the presence of formazan granules in the cytoplasm. Blood plasma viscosity was determined with the help of a conventional viscometer.

RESULTS

Analysis of blood from 15 donors wihtout any evident pathology showed that 10 min after addition of luminol the intensity of radiation in 10 cases was no more than 2-3 times of the background level, in 3 cases there was a gradual increase in the intensity of luminol-dependent CL (LDCL) that reached 5-7 background values, and in 2 cases the intensity rose to 10-15 background values immediately after the addition of luminol, after which it did not change. When luminol was added to the blood of a patient with stable angina pectoris of II or III degree, in 16 of 25 cases the intensity of radiation increased immediately to the values which were greater than the background level by tens or hundreds times; in 5 cases it grew during 10 min to the 20-30 background values; and in 4 cases it exceeded the background level by no more than 5 times. In patients with initially high level of LDCL it decreased after 3 sessions of laser therapy; however, in some cases it grew after subsequent sessions. When the initial intensity of LDCL was low, it was increased during laser therapy. Generally, the state of these patients did not improve.

Below are given some cases. Patient M. (male,63 years) had myocardial ischemia, stable angina pectoris of III functional class, and I degree circulatory insufficiency. The addition of luminol to blood of this patient taken before the therapy produced a drastic increase in the intensity of CL (Fig. 1). After 3 sessions of laser therapy, the level of LDCL significantly decreased, although shaking of the blood sample

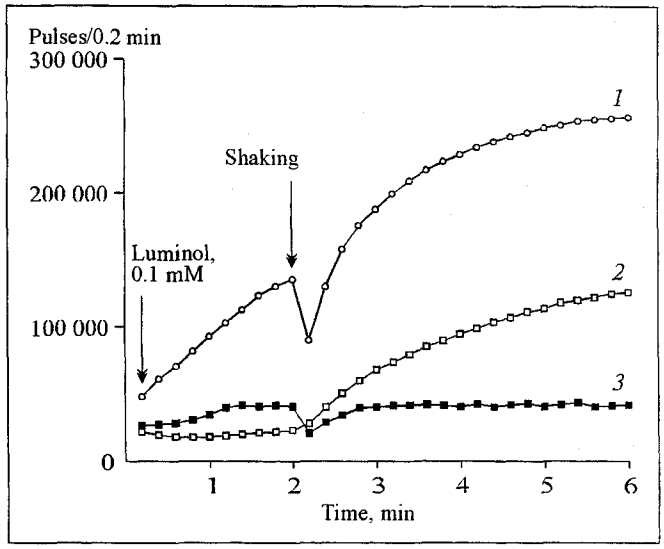

Fig. 1. Luminol-dependent chemiluminescence of blood taken from patient M. (*1*) prior to and after (*2*) 3 and (*3*) 8 sessions of laser therapy.

provoked its rise. There were no marked changes in LDCL dynamics after 8 sessions in comparison with that after 3 sessions. However, after 8 sessions shaking did not lead to extra rise of CL. At this period, the state of the patient was assessed as good. When this patient came into clinic 10 months later for a scheduled course of laser therapy, the intensity of LDCL in his blood did not exceed 2 background values. It slightly increased after 3 sessions, and after 9 sessions the intensity of CL became 25 background values 8 min after the addition of luminol to blood sample (data not shown). During the period of laser therapy this patient had a light attack of angina pectoris. In

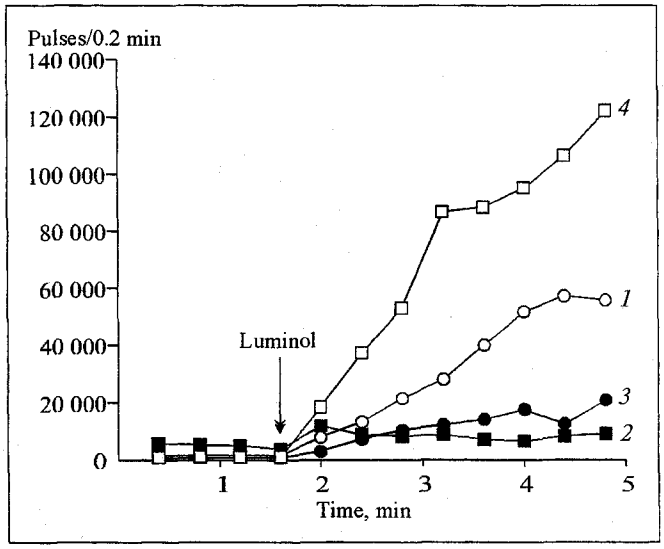

Fig. 2. Luminol-dependent chemiluminescence of blood taken from patient C. (*1*) prior to and after (*2*) 3, (*3*) 7, and (*4*) 10 sessions of laser therapy.

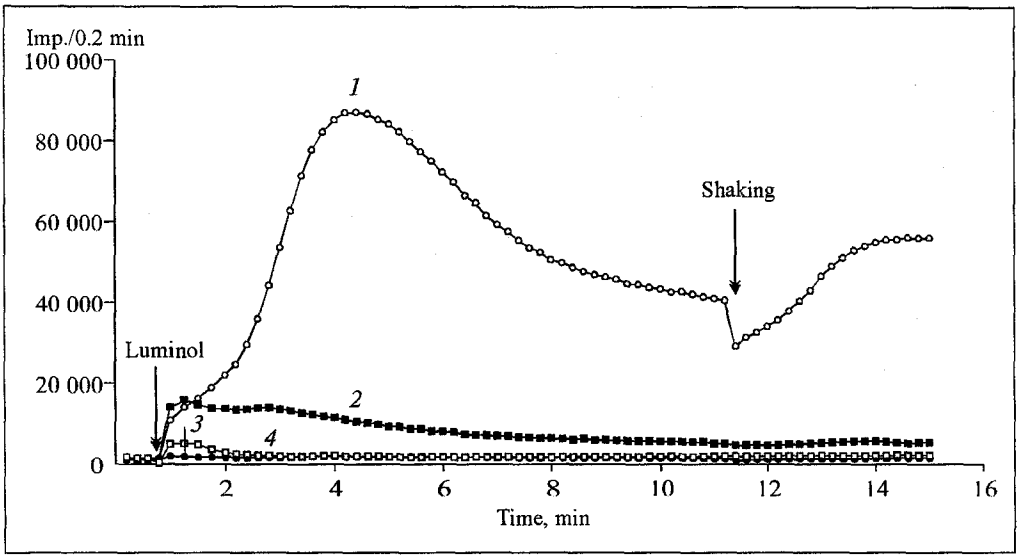

Fig. 3. Luminol-dependent chemiluminescence of blood taken from patient T. (*1*) prior to and after (*2*) 3, (*3*) 7, and (*4*) 10 sessions of laser therapy.

our study we encountered two other similar cases, so in the following the laser therapy was not administered to patients with low LDCL level of undiluted blood. These patients received conventional supporting medicamentous treatment.

Patient C. (male, 73 years, stable angina pectoris of III functional class) was discharged in satisfactory condition after 10 sessions of laser therapy, although the diagnostic pattern remained virtually the same. In this patient, LDCL decreased markedly after 3 and 7 sessions, but after 10 sessions the intensity of LDCL was higher than before therapy (Fig. 2).

The first laser therapy course for patient T. (female, 69 years, who had a similar diagnosis as patient M.) consisted of 10 sessions. The dynamics of LDCL after 3, 7, and 10 sessions was similar to that shown in Fig. 2 (patient C.). Patient T. was discharged in satisfactory condition. After 6 months, she received another course of laser therapy. This time the LDCL intensity decreased almost 10-fold after 3 sessions, and after 7 and 10 sessions the decrease was even more pronounced (Fig. 3). The patient was discharged in good condition.

Comparison of total blood count with CL-analysis data revealed satisfactory correlation in every patient between the changes observed during the therapy course in the plasma viscosity, NBT index, CAI, and CL intensity. Table 1 shows two examples of such a comparison in patient M. (good result after the first course) and for patient C. (satisfactory result of laser therapy). In patient M. the therapy resulted in a decrease of NBT index and CAI which reflect the activity of neutrophils, and it correlated with changes in LDCL intensity (Fig. 1). Plasma viscosity rose initially, but then it decreased to a lower level than prior to laser therapy. In patient C. the NBT index did not vary in a consistent way during the therapy, while a stronger correlation was established between changes in LDCL intensity and plasma viscosity (Fig. 2).

At present, intravenous laser therapy based on low-intensity He-Ne lasers is widely used to correct a large variety of dysfunctions [1]. However, the therapeutic mechanism of low-intensity laser radiation is obscure [9]. In clinical practice the parameters of laser therapy (intensity of radiation, number and duration of sessions, and intersession period) are

TABLE 1. Blood Count in Patients M. and S.

Patient	Number of laser therapy sessions	NBT test, %	CAI	Plasma viscosity, arb. units
M.	Prior to therapy	48	1.46	1.5
	3	20	0.50	1.8
	8	2	0.04	1.2
S.	Prior to therapy	37	1.25	1.9
	3	41	0.97	1.5
	7	35	0.80	1.5
	10	37	0.86	2.1

often chosen empirically. A simple, quick, and objective test is needed to evaluate the individual reaction of a patient to laser therapy. To perform the LDCL analysis of undiluted blood from patients with ischemic heart disease, only a 0.2-ml blood sample is needed without any processing. This analysis takes no more than few minutes.

A drastic increase in radiation provoked by luminol in blood of most patients attests to the presence of reactive oxygen species, such as $ClO^{\cdot}$ [4]. A decrease in radiation during the course of laser therapy indicates a decrement of the steady-state level of these species. The mechanism underlying the decrease in the level of reactive oxygen species on the next day after intravenous irradiation of circulating blood, while a similar irradiation of extracorporal blood activates neutrophils and increases the level of reactive oxygen species [3], remains unclear and requires special investigation.

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