Is there a stimulation of blood microcirculation at Low Level Laser Irradiation?

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ABSTRACT

In 1980-2000 besides the laser surgery an intensive evolution of Low Level Laser Therapy (LLLT) had started in medicine, especially in Russia as well as in several other East-European countries. At the same time the biophysical mechanisms of LLLT are still the subject of disputes. One of the most popular clinical effects at Low Level Laser Irradiation (LLLI) being mentioned in medical publications for justification of the LLLT healing outcome is a stimulation of blood microcirculation in irradiated area. It was declared *a priori* at a dawn of LLLT and is now a basis of medical interpretation of healing mechanisms of LLLT at least in Russia. But in past 20 years a lot of investigation was carried out on optical registration of microhaemodynamic parameters *in vivo* as well as a number of noninvasive diagnostic tools was created for that. So, today it is possible to experimentally check the blood microcirculation stimulation hypothesis. Our study was aimed on that during the past 10 years. The most precision and accurate experiments we have carried out recently using simultaneously three different noninvasive diagnostic techniques: Laser Doppler Flowmetry, Tissue Reflectance Oximetry and Infrared Thermography. All these methods didn't confirm the effect on the blood microcirculation stimulation in skin or mucosa at irradiation with the power density below 50 mW/cm^2 and irradiation time up to 5-6 minutes. Above this threshold the heating on 0,8…1 ^{0}C of tissue in the field of irradiation and the corresponding synchronous increase of all parameters of microhemodynamics were observed.

Keywords: Laser, low-intensity, therapy, stimulation, blood, microcirculation, diagnostics, spectrophotometry

1. INTRODUCTION

In the modern medicine, especially in Russia as well as in a number of other East-European countries, different physiotherapeutic methods of treatment and prevention of diseases are widely used now. These physiotherapeutic methods cover application of various natural or artificially created healing physical factors of mechanical, thermal or electromagnetic nature. In the past 20-25 years special area among them was occupied by low level laser radiation (LLLR). Therefore, in 1980-2000 besides the laser surgery the intensive evolution of Low Level Laser Therapy (LLLT) had started in medicine, especially in Russian physiotherapy, as a modern continuation of a sunlight therapy technique[1,2]. At the same time, the biophysical mechanisms and a real clinical effect of LLLT are still the subject of disputes. Today one may speak of at least 4-5 initial mechanisms of LLLT: thermal effect (heating of tissue), photodynamic one (activation of singlet oxygen), photochemical effect (direct photo-destruction of organic molecules), placebo effect and so on. Meanwhile the healing effect of LLLT is far not always reproducible or guaranteed. There is in publications a lot of inconsistent information concerning LLLT. Take just speculations on dosage determination in LLLT! Recommended settings for the useful power density in different guidelines differ in hundred times or more[3] (from 0,5 up to 200 mW/cm^2), and the recommended "dose" (energy density) ranged from 0,1 up to 120 J/cm^2. Moreover, the World Association for Laser Therapy (WALT) had formulated some recommendations on effective doses for LLLT by 2010 only, and only for wavelengths of 780-860 nm (continuous or pulsed mode) and 904 nm (pulse mode)[4]. But the recommended doses are still in a very wide range, 1…6 J per area (six times!) of irradiation at a power density of more than 5 mW/cm^2. Thus, the problem is opened yet.

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In our opinion, one of the reasons of such situation in LLLT is an insufficient application of objective methods for direct and quantitative registration and visualization of the direct therapeutic effect during each LLLT procedure on both a local and a systemic level (blood microcirculation system, cell metabolism, basic parameters of homeostasis, etc.). For example, the stimulation of blood microcirculation is described in most Russian medical articles as one of the basic responses on LLLR or one of the basic positive biological effects of LLLT[5-8]. And most authors are confident in this case that no substantial heating of the tissues occurs at the LLLT procedures, i.e. the temperature of the irradiated tissues doesn't increase by more than 0,1 ^{0}C (cold therapy)[4]. But these conclusions were obtained about 10–15 years ago on the basis of ordinary clinical observations and of results of morphological studies. At that time there were no devices available to physicians to record microcirculation processes and the dynamics of the surface temperature of biological tissues with sufficient accuracy and in real time, so the direct measurements were not done. Today such devices have appeared. These include, most importantly, modern noninvasive spectrometric instruments that make it possible to monitor the tissue respiration and perfusion of tissues with blood[9] as well as apparatus for digital infrared thermography and the thermal-vision monitoring devices that record the temperature over a large surface area of the body within $\pm$0.05 ^{0}C accuracy and with the same sensitivity[10,11]. All these facts opens up the prospect of direct and on-line checking of made earlier conclusions on biostimulation of blood microcirculation at low level laser irradiation (LLLI).

Our study was aimed on that during the past 10 years. The more precision and accurate experiments we have carried out recently using simultaneously three different noninvasive diagnostic techniques: Laser Doppler Flowmetry (LDF), Tissue Reflectance Oximetry (TRO) and Infrared Thermography (IRT)[11,12]. In this paper we offer for discussion our main last results summarising them.

2. MATERIALS AND METHODS

Forty five people took part in the last study. They included 10 nominally healthy volunteers (in that number including both authors of the article) and 35 patients of the "MONIKI" Clinics suffering from various skin and mucosa blood microcirculation disorders, who, according to various indicators, had been prescribed to receive planned procedures of superficial LLLT or intravenous laser irradiation of the blood (ILIB). All subjects were chosen with normal or spastic types of blood microcirculation, in order to prevent the hyperemic type of microcirculation from affecting the experimental results[9]. Of the total number, 20 of the subjects were irradiated by LLLR on a surface of different skin zones of upper extremities (the forearm, palm, and wrist), 15 patients as well as 2 volunteers received ILIB and 15 subjects (8 patients and 7 volunteers) were irradiated by LLLR on a surface of the mucosa of oral cavity. Three volunteers were also tested using ordinary contact thermoelectric methods of skin heating. Ten "quasi-placebo" measurements were made in upper extremities with no irradiation to obtain a set of data on the parameters of microcirculation and oxygenation of blood in a state of the rest (both for healthy volunteers (8) and for patients (2)).

All parameters of blood microcirculation – an index of microcirculation I_m (blood flow, perfusion units, [PU]), tissues saturation of oxyhaemoglobin S_tO_2 (%) and blood volume V_b (%) were simultaneously and *in vivo* recorded by the commercial laser multifunctional noninvasive diagnostic system LAKK-M[13] (Lazma Ltd., RF) as well as by laser Doppler flowmeter LAKK-01 (in a number of experiments). An IRTIS-2000ME digital medical thermograph (Institute of Radio Engineering and Electronics, Russian Academy of Sciences) was used to measure the surface temperature and to obtain thermal images. An external thermal heater from the LAKK-01 apparatus with the options of stabilization and independent monitoring of the heater temperature during the heating procedure was used in experiments with heating as a thermal probe (heating probe). The heater temperature was set and maintained at a level of 41.5–42 ^{0}C. The time of skin heating was 3,5 min.

Two types of commercial laser therapeutic apparatus with different designs were used for superficial LLLI: the ULAN-BL-20 (pulsed irradiation regime (PW), λ=890 nm, pulse-repetition rate 30 kHz) and the ULF-01 (continuous irradiation regime (CW), λ= 632 nm, max output power 20 mW). Only the continuous irradiation regime with the use of ULF-01 was used for ILIB. The area of the skin or mucosa irradiated zone was varied within the limits 0.3–4 cm^2. To attempt to find how the thermal effect depends on the power density, part of the experiments on two volunteers were especially done with three different irradiation zone gradations along the spot diameter (0.3, 1.5, and 3 cm). The output power of the LLLR from the needle tip during ILIB was 2 mW. In this case, a needle with a lightguide was introduced into an ulnarvein of the patient (Figure 1), while a fiber optical probe from the diagnostic equipment for measuring the blood microcirculation parameters was fixed on the distal phalange of the third finger of the same arm. This region is chosen as having the richest network of capillaries and arteriovenous shunts, and it quickly and strongly reacts to any changes in the ambient conditions[6]. The duration of the LLLT procedure was varied from 5-10 min for superficial procedures (on skin or mucosa) to 20 min for ILIB. In the last case, all diagnostic parameters were recorded both before beginning the irradiation (background recording) and simultaneously with the irradiation and then, also, for several minutes after it ended. All the measurements were made in the same office at room temperature (21–23 ^{0}C) by the same group of researchers. Before any procedure was begun, an examined subject was made to sit still for 5–7 min to normalize his blood flow. Totally, about 70 different experiments and measurements were carried out as part of the studies described here. The total distribution of the subjects on experimental procedures is presented in Table 1.

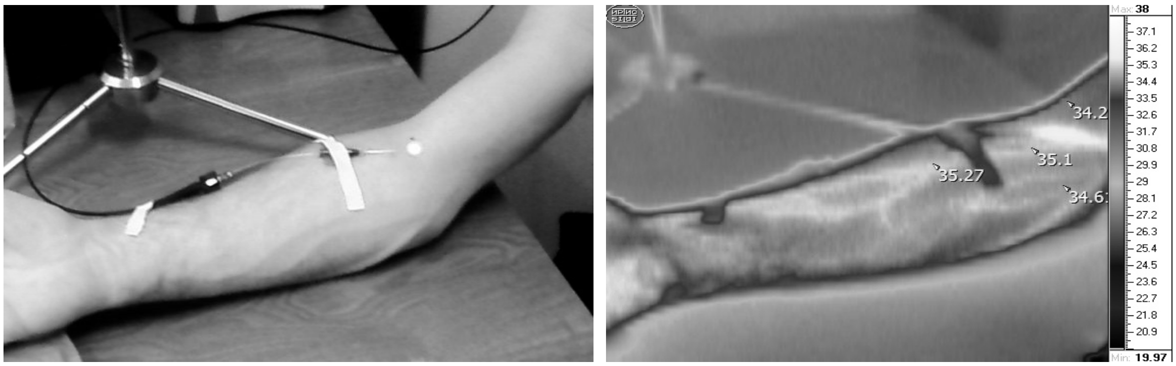

Figure 1. Intravenous LLLT procedure (left) and the thermal image of the irradiated zone (right).

Table 1. Total distribution of the subjects on experimental procedures.

Subjects	**Placebo**	**ILIB**	**Skin LLLT**		**Mucosa LLLT**		**Thermo**
		CW	CW	PW	CW	PW	
Volunteers* (10)	8	2	5	5	5	2	3
Patients (35)	2	15	5	5	6	2	-

* A number of volunteers have passed through examination several times.

As an example, the experimental design for cutaneous LLLT and the method of fixing the diagnostic fiber probes of the LAKK-M and LAKK-01 systems on skin are shown in Figure 2 along with a thermal-vision image of the tested region. The thermal-vision image, from which the temperature was subsequently automatically calculated, covered the entire area of the irradiated surface and the surrounding region in which the laser radiation acted on the tissues. This made it possible subsequently to analyze the dynamics of the temperature changes not only directly at the center of the irradiation spot but also along its periphery and the unirradiated tissues that surround it. The similar setup was used at oral cavity irradiation. In the case of the skin heating by the external LAKK-01 heater the skin temperature was registered and determined on the image close to the edge of the heater.

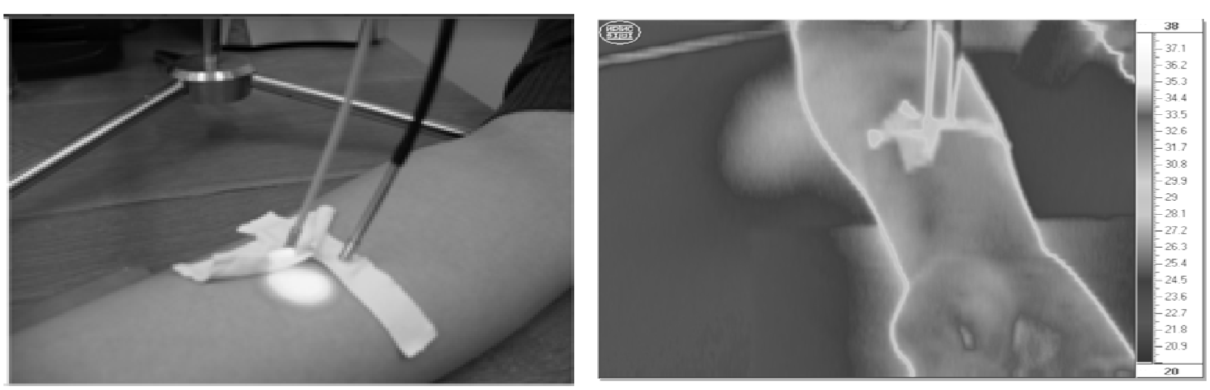

Figure 2. Skin irradiation and diagnostic procedure (left) and the thermal image of the irradiated zone (right).

3. RESULTS AND DISCUSSION

Examining the correlation of the blood-microcirculation parameters (I_m, V_b, S_tO_2) and the surface temperature of the tissues (T), all the trends obtained in different experiments can be conventionally divided in their dynamics into three main (typical) groups:

I. Within the limits of accuracy of the measurements (5-8%) there are no visible dynamics and no changes in all parameters during the entire experiment, regardless of the presence or absence of external LLLI.

II. The differently directed drift (oscillations) of the values of I_m, V_b, S_tO_2 within its variations of $\pm$10% from the mean and of T within $\pm$0.5 ^{0}C during the entire experiment, with no unambiguous or coordinated dynamics between all parameters, also regardless of the presence or absence of external LLLI (Figure 3).

III. An assured simultaneous increase of I_m, V_b, S_tO_2 and T, more, than for 10% and for 0,8 ^{0}C respectively, during the entire experiment when there is external action (irradiation by LLLR or thermal heating) and a latent period and/or subsequent falloff after the action ends (Figure 4).

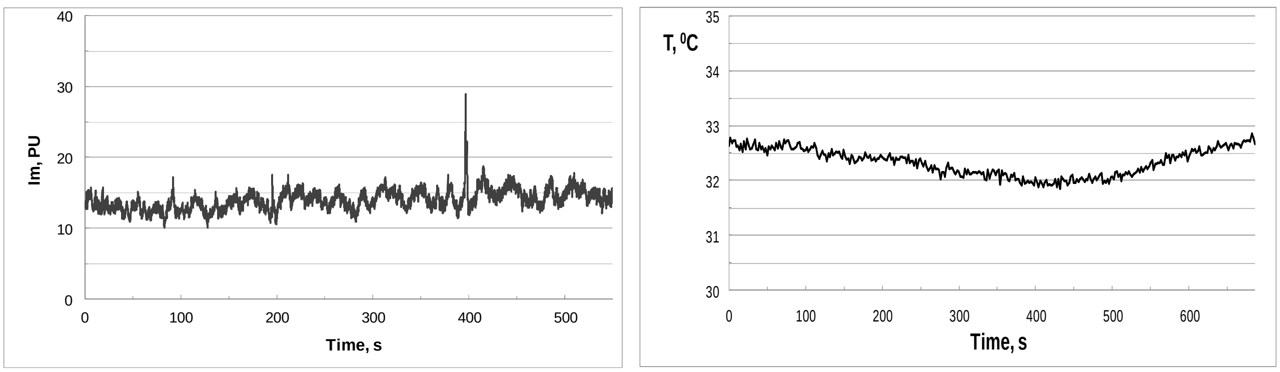

Figure 3. Natural oscillations in I_m and skin temperature T without coordinated dynamics between them during the same procedure. Beginning of the LLLI: 50 s; end: 550 s.

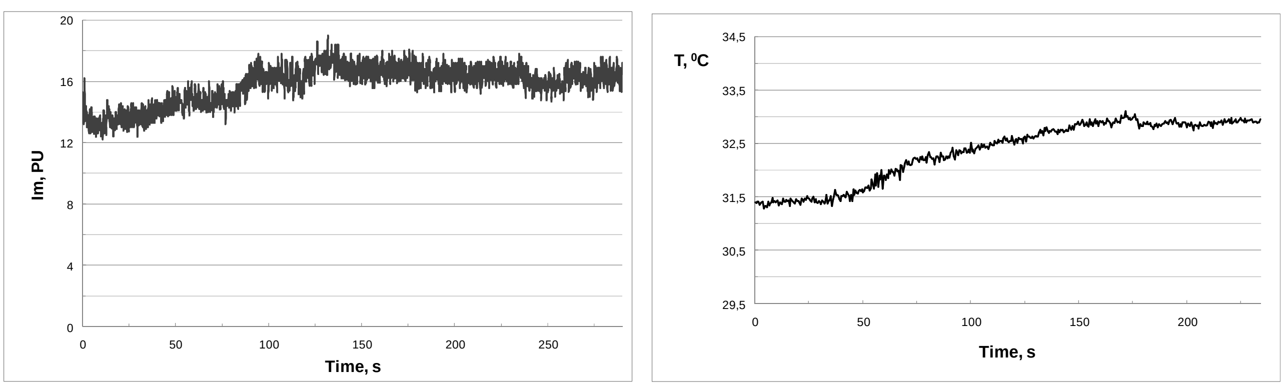

Figure 4. Visible simultaneous dynamics of the microcirculation index I_m and of the temperature T accompanying intravenous laser irradiation of the blood of an oncologic patient for 240 s. Beginning of the ILIB procedure: 10 s; end: 200 s.

For the group II it was impossible during our experiment to detect any unambiguous correlation in the behavior of the microcirculatory parameters I_m, S_tO_2 and/or V_b with the dynamics of the temperature T. This was independent of whether a sick or healthy subject participated in the experiment, whether or not there was LLLI in the experiment, whether there was cutaneous (mucosa) irradiation or ILIB, or whether the LLLI was continuous or pulsed. Each of the recorded diagnostic parameters in all these situations most likely behaved independently from other parameters manifesting natural fluctuations of the parameters in the group. That is, in this group all changes (or their absence) in parameters are probably caused not by external action – superficial LLLI or ILIB - but were determined by ordinary physiological rhythms and the background physiological variability of the living system, since this variability was observed in six out of ten cases of experiments without any irradiation, i.e. in “quasi-placebo” experiments (one case for a sick subject and five for healthy subjects). Generally, our experiments showed that when the changes are small, on the level of physiological variations or a little higher, the changes of the surface skin temperature do not always correlate with changes of the perfusion of tissues with blood. This can be evidence that not all thermal processes in skin are determined only by the blood-microcirculation parameters. Some thermal fluctuations apparently depend on other processes, for example, on the process of diffusion and evaporation of moisture from the skin surface.

On the average, although some prominence of the reaction of the microcirculatory system and the skin temperature was observed in the zone of superficial laser irradiation and during ILIB (see Figure 4, for example), this was true in no more than 20% of the cases (12 cases in a total) of LLLI with ordinary therapeutic doses of irradiation. Meanwhile, in 80% of cases, it was impossible in general to detect changes of the temperature or of the blood microcirculation and blood oxygenation parameters during LLLT procedures on the background of natural physiological fluctuations of all parameters like it is presented in Figure 3. And this behavior was manifested in a wide range of power densities and irradiation times. Moreover, even in the 20% of the positive cases indicated above due to diagnostic data fluctuations during the LLLI, majority of changes were all just barely noticeable on a background of these strong physiological fluctuations. Only 3 cases, excepting experiments with heating had strong manifested results. However, everywhere when the perfusion I_m varied evidently during LLLI procedure, the surface temperature in the irradiation zone changed at the same time and in the same direction. The opposite was not observed.

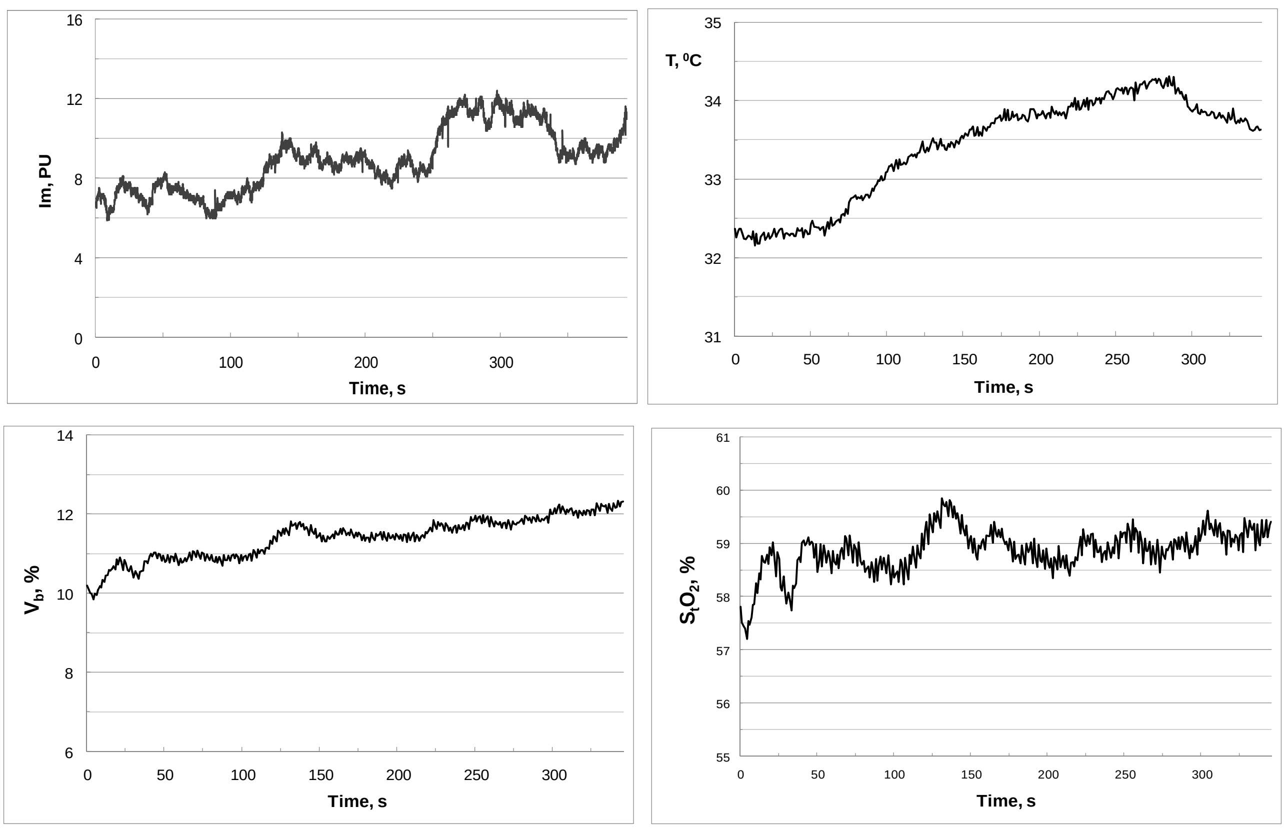

Figure 5. Obvious simultaneous dynamics of all parameters accompanying contact heating of the wrist in the norm. Beginning of heating: 60 s; end: 270 s.

Unambiguous and correlated dynamics of all four parameters (S_tO_2, V_b, I_m, and T) were stably detected only with strong enough action, associated either with large irradiation power densities (for example, starting from 250 mW/cm^2 for cutaneous irradiation at λ=632 nm or from 50 mW/cm^2), or when the tissues are directly heated with a thermal heater (Figure 5). Moreover, the dynamics was identical for LLLT procedures and for ordinary heating. So, the term "low-intensity" here is evidently no longer quite correct. In all cases of obvious, visible reaction of the microvascular system to the external action, the temperature in the region of action had increased from the initial level by at least 0.8–1,0 ^{0}C. Similar dynamics with lower power densities of irradiation were observed only with external irradiation of the mucosa or the skin by continuous laser radiation in areas having increased thermal sensitivity. For instance, the dorsal side of the radiocarpal joint of one of the subjects began to heat up and responded to the action by obvious vasodilatation at a power density of about 50 mW/cm^2. In this case the temperature increasing was around 1,2…1,3 ^{0}C together with some synchronous and correlated increase of all other parameters of blood microcirculation (I_m, S_tO_2 and V_b). Consequently, the leading mechanism of LLLR action is also a thermal mechanism in this case.

One more interesting result was obtained at analysis of rhythms of microcirculation parameter I_m fluctuations before and after irradiation for cases when the increase in I_m during the course of LLLI was observed. It was done by the conventional wavelet analysis of the index I_m before and after LLLT procedure. Figure 6 shows the typical results for several cases observed.

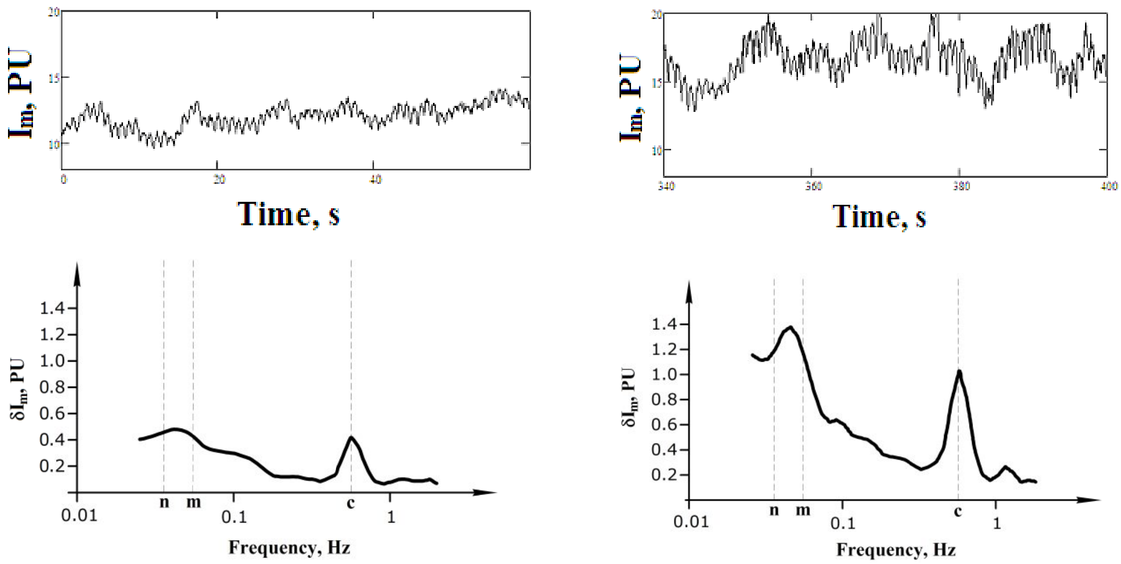

Figure 6. Examples of typical fragments of LDF-grams (above) and their wavelet analysis (below) before (left) and after (right) laser irradiation: n – neurogenic rhythms; м – myogenic rhythms; c – cardiorhythms

On these schedules it is possible to see distinctly visible increase in the blood flow (in I_m) from around 12…14 PU (in average) up to 15…18 PU (increase of around 25…30% from the initial level) and appreciable increase in a contribution of neurogenic and myogenic mechanisms in the blood microcirculation regulation. However, we have to say, that such picture with so enhanced I_m was found out in one of our superficial LLLI experiments only. All other experiments showed essentially smaller changes in all parameters of microhaemodynamics. Nevertheless, the enhanced neurogenic and myogenic fluctuations observed after the LLLT procedure could be accepted as a manifestation of a real effect of biostimulation as well. Moreover the LLLT output in the form of enhanced regulatory rhythms in the system of blood microcirculation don't has necessity to be correlated with the increase of the skin temperature or with other microhemodynamics parameters, that can be very interesting.

But it would be true if the similar effect wasn't observed in our placebo experiments. For example Figure 7 presents a natural fluctuation in the index of microcirculation I_m (blood flow) at the absence of any action on the hand's skin in norm. In this placebo test I_m was registered during 16 minutes from the palmary surface of skin of a third finger distant phalanx while the hand motionlessly lay at the level of the examinee's heart. On the graphics the increase in amplitudes of low-frequency fluctuations of a blood flow after 200 seconds of the record in an explicit form is visible. It can be a consequence of a small hypostasis of a hand in a motionless condition as well as of an attempt of the examinee's organism to compensate a lack of blood outflow by the increase of low-frequency vasomotion of small vessels of a microcirculatory bed. I.e. the blood microcirculation system is very variable and also is very adaptable under any external influences, so the direct interpretation of changes of its parameters is not always possible in terms of the induced changes owing to only influence of LLLR. Therefore only comparative experiment for enough big groups of examinees with influence of LLLR and without one can answer to the question what prevails in these cases - changes from hypostasis in the conditions of limited mobility of an extremity, or direct effect of laser radiation. We consider true the first. We have carried out similar 10 placebo experiments. The results expressed in number of cases of parameters changes are presented in Table 2. Comparison of data was carried out for the first and the last third of the I_m records which in all experiments was lasting 16-20 minutes.

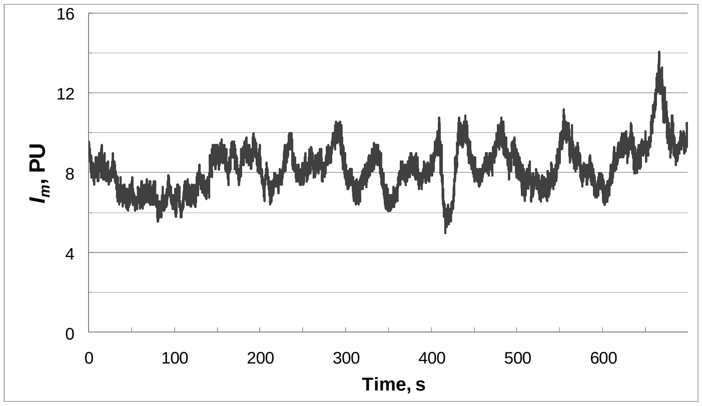

Figure 7. Natural fluctuations of the blood flow (I_m) at the absence of any impact at placebo experiments.

Table 2. Results of placebo experiments on changes of I_m and its rhythms of fluctuations during records.

Parameters	**Considerably raised, cases**	**Considerably went down, cases**	**Didn't change, cases**
Perfusion I_m	5	1	4
Neurogenic amplitude	3	2	5
Miogenic amplitude	4	2	4

Thus, at placebo experiments I_m can have physiological fluctuations similar to the fluctuations observed at several our experiments with LLLI. It confirms the assumption of natural character of the phenomenon.

The results of our studies on the whole thus confirmed the latest experimental results of Ref.[14] that variations of the parameters of the microcirculation and oxygenation of the blood under LLLT occur much less often and are less clearly expressed (if they are there at all) than reported by purely medical primary publications. A pronounced reaction in which microcirculation increases under the LLLI shows up only in the case of fairly powerful and prolonged irradiation, when ordinary thermal heating of the tissues becomes the leading cause, and the reaction itself of the microvascular system becomes identical to the reaction to a contact thermal probe. Any pronounced changes of more than 10% in microcirculation happen only when tissues heating happens as well. In all experiments in which the microcirculation in tissues clearly raised during LLLT, the thermograph also clearly recorded raising of tissues' temperature greater than 0.8 ^{0}C. Less pronounced reactions within the limits of 1–10% of the initial values of the I_m, if they occur, are fundamentally "drowned" in the natural physiological fluctuations of the parameter and cannot be reliably recorded nor be taken seriously in the aspects of the mechanisms of LLLT action. This reaction probably does not exist in most cases.

Can this be true in principle if there are a lot of publications on the stimulation of microcirculation accompanying LLLT? Apparently it can. Quite recently, Plavskii et al.[15] indicated that LLLT was most widely used only in Russia and the countries of the former USSR, whereas, abroad, in particular, in USA, Great Britain, Canada, and many other countries, there is extremely cool reception to it, especially to ILIB. The authors analyzed about 3000 professional publications on LLLT, but they found methods of conducting the studies that satisfy criteria convincing to a physician (randomized clinical tests) in only about 140 of them. Only a few publications contained a description of an experimental technique with double-blind control, in which neither the physician nor the patient knew whether the laser was operating. In this case, in half of the papers which report such monitoring it is pointed out that the results of LLLT are indistinguishable from the placebo effect. A Japanese publication[16] is instructive in this sense and has existed for a long time but has apparently remained unnoticed by medical specialists. It reported the action of LLLI of different wavelengths and power densities on the vascular tone in the experiment with individual segments of the vessels. For comparison, the reaction of the vessel to ordinary heating was also tracked. The fundamental conclusion of the article is that the vascular tone reacts only to heating, regardless of by what method it is obtained - by contact heating or by means of LLLI. In all cases of irradiation and contact heating, a reaction of the vessels was observed in the experiment only when the temperature in the zone of action increased by about 1 ^{0}C from the initial level. Neither contact heating nor LLLI below this threshold produced an appreciable change of the vascular tone, and this is consistent with our results.

4. CONCLUSION

In this study, for various procedures of LLLT the temperature fields on skin and mucosa surface and the blood microcirculation parameters in the skin and mucosa were simultaneously measured by means of the IRTIS-2000ME digital thermograph and the LAKK-M spectrophotometric diagnostic system. The results showed that all three diagnostic real time methods – Laser Doppler Flowmetry, Tissues Reflectance Oximentry and Infrared Thermography, used in the study, - didn't confirm that LLLI has unequivocal stimulating effect on the blood microcirculation system in skin or mucosa at irradiation with a power density below 50 mW/cm^2 and irradiation time up to 5-6 minutes. Above this threshold the heating on 0,8…1 ^{0}C of tissue in the field of irradiation and the corresponding synchronous increase of all parameters of microhaemodynamics were observed authentically. So, today we have a quite proved opinion that the heating is the dominant mechanism of the LLLR actions on the blood microcirculation system.

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