PREOPERATIVE ACTIVATION OF THE IMMUNE SYSTEM BY LOW REACTIVE LEVEL LASER THERAPY (LLLT) IN ONCOLOGIC PATIENTS : A PRELIMINARY REPORT

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In vitro cellular and *in vivo* animal studies have pointed to the possible boosting effect of Low reactive-Level Laser Therapy (LLLT) on the autoimmune system of immunodeficient cancer-inoculated animals, resulting in an increase in the expected life-span of the irradiated animals. Following such studies, the authors designed a study to evaluate the effect of LLLT as an adjunctive therapy for conventional surgical intervention in cancer in man. A comparative study of different types of irradiation from low incident energy level lasers was performed on 60 oncologic patients, irradiation being delivered during the immediate preoperative period. External irradiation with a semiconductor laser (wavelength 890 nm); internal irradiation with a helium-neon laser (wavelength 632.8 nm); and a combination of both methods was applied. The most effective irradiation was the external one made with a semiconductor laser. Studies were carried out on white cell components in blood, assays of immunoglobulin activity (IgA, IgM and IgG) were made, in addition to the determination of the behaviour of T-lymphocyte fractions (active roseate T-cells, T-helpers and T-suppressors) post LLLT. It was seen from the data that the total immunoresponse actually increased following LLLT, with no visible increase in tumoural remnant size. Although more detailed qualitative experimental and controlled work must be done before this application of LLLT can be carried out on a regular basis, the authors feel strongly that in this preliminary report, the findings point to an exciting and possible use for LLLT, in particular for the photoactivation of the autoimmune system and tumoural antigen photomodification, and in general for the treatment of immunodeficiency.

KEY WORDS Low level laser therapy LLLT Photoimmunological reaction Oncology Photo-immunoactivation effect Postoperative complications

Introduction

Laser irradiation has found a wide application recently in various fields of medicine. Laser therapy is especially developing rapidly.¹ Bearing in mind that one of the possible actions of therapeutic doses of laser energy, as distinct to the photodestructive reactions in laser surgery, is to inhibit or even retard unlimited cell division in tissues as seen recently in the literature,² we tried to affect the whole organism as demonstrated by a spectrally-selective irradiation of oncopatients, using low incident energy lasers, in what is now increasingly being referred to as low reactive-level laser therapy or LLLT. Laser therapy was used in combination with conventional surgery, delivered immediately prior to the surgical procedure.³

This technique of treatment has been supported by experiments performed earlier, which have shown

that: (1) LLLT activates the immunocompetent aspect of cells.⁴ (2) Laser irradiation can modify tumoural cells up to and including destruction, without increasing their temperature;⁵ in other words, a pure photobioactivative response. (3) The irradiation of experimental tumours leads to the increase in animal life-span as well as to the retardation of tumoural growth.^{6,7}

The aim of this study is to describe the results of our clinical investigations on laser therapy in oncological patients with different tumoural localization. The authors have also tried to give an up-to-date theoretical background of the mechanism of the photobioactivative effect of LLLT on cells, based on existing data as well as on the findings of the present study.

Methods of Investigation

Comparative white blood count (leucocytes, lymphocytes, monocytes) for the different methods of laser

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delivery was carried out by the method of direct calculation under a microscope in Gorjaiv's chamber. Immunoglobulin components, IgA, IgM, IgG, in blood serum were determined on the Ps-9 (Labsystems) analyser in the laboratory of the Regional Clinical Hospital in Rjazan. T-lymphocytes were assayed according to Hasnill. The determination of T-lymphocytes, T-helpers and T-suppressors was done by standard techniques.²⁰

Patients were cytologically and histologically diagnosed in the Regional Oncohospital, Rjazan. Laser irradiation and surgical interventions were performed at surgical departments of various city hospitals.

Results of Clinical Investigations

LLLT was performed in 60 oncopatients in the immediately preoperative period.

In 45 patients, external irradiation of definite zones, (tumoural projections, lymph nodes) was performed with infrared (IR) semiconductor lasers with a wavelength of 890 nm. Three patients underwent intravenous irradiation with He-Ne laser, wavelength 632.8 nm. Twelve patients had the external IR irradiation in combination with the intravenous He-Ne laser irradiation. Table 1 shows the distribution of oncopatients and the type of irradiation.

When comparing results it was found out that the most effective method was the external irradiation with semiconductor laser. We also found out the optimal parameters which produced the most marked effect with the least postoperative complications.

The dynamic analysis of white blood elements has shown that the number of leucocytes increased by 5.7%, 5.6% and 5.5%, respectively, following external, combined and internal LLLT (Figure 1a). Blood lymphocytes after external irradiation increased by 5.5%, but after the combined and intravenous irradiation they showed changes of

0.4% and 5.6%, respectively, below their initial level (Figure 1b). Monocytes after the external irradiation increased by 2.4%, by 0.4% after the combined one, and by 20% after intravenous one ($p < 0.5$) (Figure 1c). Complete protein after the external irradiation increased by 4.6%, but decreased below the initial level with the combined and intravenous irradiation (data not shown).

After the external IR-irradiation IgA was markedly increased (by 43%), and still remained high 4–5 days after the operation (Figure 2a). After the combined irradiation IgA increased slightly, decreasing to the initial level in the postoperative period. The results after the intravenous therapy were in the intermediate position (Figure 2a).

The dynamics of immunoglobulins of class M (Figure 2b) showed that after the external irradiation they increased slightly at first, but by the 4–5th postoperative day they increased sharply. After the combined irradiation we marked their progressive decrease (Figure 2b).

IgG increased after the external and intravenous irradiation; then by 4–5th postoperative day it decreased; following the external irradiation this decrease was markedly below the initial level (Figure 2c).

In a group of patients who underwent radical surgeries (stomach resection, gastrectomy) we investigated T-cell immunity after the external IR irradiation. After laser therapy the number of active T-lymphocytes increased by $49 \pm 3\%$, maintaining this level of increase by 4–5th postoperative day (Figure 3a). The number of T-helpers after laser therapy increased by more than twice showing a tendency to further increase at the end of our examination (Figure 3b). However, T-suppressors constantly decreased (Figure 3b).

Stomach Cancer

Of 14 patients with established stomach cancer (aged 39–66) who had palliative surgeries (Table 1),

Table 1. Distribution of cancer sites and radiation types

Tumour	External irradiation	Intravenous irradiation	Combined irradiation
Stomach cancer	19	—	—
Colon and rectal cancer	5	3	11
Mammary gland cancer	14	—	—
Skin melanoma	3	—	—
Kaposi's carcinoma	1	—	—
Renal cancer	—	—	1
Oesophageal cancer	1	—	—
Thyroid gland cancer	1	—	—
Lymphogranulomatosis	1	—	—
Total	45	3	12

Figure 1. Changes in concentration of white blood cells after laser therapy. (a) Leukocytes. (b) Lymphocytes. (c) Monocytes

11 patients had an early restoration of peristalsis on the 1–2nd postoperative day. One more patient had mild pancreatitis which disappeared by the 3rd day; during the surgery his spleen was injured. One patient had a mild pneumonia in his postoperative period which was totally controlled by the sixth day. In the group of eight patients who underwent the most effective laser therapy, there were no complications.

Rectal and Colon Cancer

Patients with cancer of the rectum and colon were 54–70 years old (Table 1). Ten patients underwent radical operations, one patient refusing surgery. Early restoration of peristalsis (by the 1–2nd day) was noted in 11 patients. No postoperative complications were observed; six patients reported relief from their pain syndrome.

Breast Cancer

Patients with cancer of mammary glands were aged 38–72 (Table 1). Two patients showed a reduction

of the neoplasm after laser therapy; two other patients demonstrated an improvement in radiation following X-ray therapy, following laser therapy. In the one patient who refused surgery, after 10 months a control mammography revealed an increase of tumour size by 0.5 cm.

Skin Cancer

Three patients with skin melanomas showed no increase of tumours after laser therapy; there were no complications during the postoperative period.

Oesophageal Cancer

The patient with cancer of the oesophagus reported relief from associated pain, and an improvement in the ability to manage food.

Negative Reactions

The patient with the Kaposi's carcinoma had mild negative dynamics. None of the other patients had any negative reaction after laser therapy.

Figure 2. Changes in immunoglobulin components during laser therapy, surgery and in the postoperative period. (a) IgA. (b) IgM. (c) IgG

Discussion

The general strategy of our work has been described³ and comprises the following:

- (1) To initiate an immune reaction against neoplastic cells, due to changes in their antigen properties, with the help of photobioactivation with irradiation by low incident levels of laser energy.
- (2) To find the optimal regime of irradiation to achieve laser-induced activation of the immune system.
- (3) In order to counteract and remove a large tumoural mass, the organism must have ample strength in the immune system to enable speedy detoxification of resultant

toxins after cell destruction: to achieve that, it is important that surgery must be performed immediately after laser therapy.

- (4) After the surgical removal of the tumour the therapist must provide conditions for developing an immune reaction strong enough to destroy the remaining tumoural cells, and to speed the process of postoperative wound healing.

These above-mentioned points have been based on preliminary experiments. First, LLLT irradiation can modify the plasmatic membrane of different types of cells;^{5, 8} second, our unpublished data show that during laser therapy of experimental tumours in rats (Worker's cancer of mammary glands) we could observe the retardation in their growth as

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Figure 3. Kinetics of T-lymphocyte fractions.
 T_{Hf}—Active rosette-formed cells.
 T_H—T-helper cells.
 T_S—T-suppressor cells

well as changes in their histological structure with signs of cell dystrophy throughout the whole tumoural mass following irradiation with an infrared (IR) laser beam. In another series of experiments, when we irradiated an inoculated Luis carcino-sarcoma in rats again using an IR-laser, we could observe a considerable prolonging of the animal's life-span by 50%.⁹ At the same time we noted that photochemical changes take place in tumour cells without any rise of temperature in the adjacent mass. When dosages of irradiation are large enough, the permeability of the cell plasma membrane is disturbed, as proved by the swelling of the cells and the subsequent cytolysis. These data have been obtained for laser-irradiated suspensions of Luis carcino-sarcoma cells and ascitic embryosarcoma when the cultures were exposed to laser beams in red and IR spectrum (628.3 nm, 762 nm).^{4, 10} Additionally, it has been proved that when human lymphocytes are irradiated *in vitro* by the helium-neon laser (632.8 nm) we note their functional activity determined by immunological tests. On analysing electron microscope data we can see changes at the superficial ultrastructure of the plasmatic membrane in irradiated cells which are characterized by a specific mace-like formation at the end of membranous microfibrils.²

Now, let us analyse how the data obtained correspond to the planned purposes of the trial. During laser therapy—under any regime of irradiation—we can observe a general increase in the number of leucocytes (Figure 1a); this may be a result of a partial migration from the sites of their

localization in the immuno-producing organs. At the same time, external irradiation is characterized by a simultaneous increase of lymphocytes in blood (Figure 1b), while internal irradiation is characterized by changes in monocyte fraction (Figure 1c). These differences could be explained by the fact that the external irradiation has a larger target area, and lymph nodes are in the irradiation zone. Whereas in blood, laser irradiation is very rapidly attenuated inside the blood vessel. In the first case, lymphocytes are predominantly activated and released into the blood flow; in the second case, laser-induced changes in blood may be forcing monocytes to migrate there. Monocytes are precursors to macrophages, however lymphocytes can themselves perform a 'killer' phagocytic function; so, it could be argued that the intravenous irradiation regime leads to a more gentle reaction with a large lag-period.

The increase in immunoglobulins of class G, which possess a clearly proved cytotoxicity to tumoural cells, indirectly shows the increase in the number of T-killers,¹¹ while the increase in T-activated lymphocytes along with a considerable decrease in T-suppressors and T-helpers arguably indicates the development of a strong photoactively-induced immune reaction. As it is shown that the antigen which is able to trigger this reaction is in these cases absent, we can conclude that the low-intensity laser irradiation can possess such a triggering effect. The peculiarity of the laser-induced primary immune reaction lies in the fact that the antibody synthesis begins from immunoglobulins G and A; perhaps also from other classes, but definitely not from I and M. At the same time it is known that the induction of foreign protein causes antibody synthesis originating from immunoglobulins of class M, which are the resultant molecules from an early immune response, and are simultaneously receptors of B-lymphocytes.¹² This difference supports the hypothesis of the direct laser activation of immuno-competent cells *in vivo* and corresponds to data *in vitro*.²

After surgery, the present study shows the amount of IgG and IgA declines sharply while the concentration of IgM increases (Figure 2). The average period taken by IgG partially to disappear from the circulating blood is about three weeks in man.¹² Thus, such a quick decline as seen in the present trial is difficult to explain only by the traditionally accepted antigen-induced response. This phase is likely to be considered as a reaction to postoperative inflammation.

Following LLLT, the total force of the immune response is certainly not weakened; quite the opposite. It becomes stronger, which is proved by a further increase of T-helpers and decrease of T-suppressors (Figure 3). However, it is not clear from this study if the developing process actually stimulates the tumoural remnants, although we can

tentatively state that this does not appear to happen.

Let us try—though our knowledge is not complete—to draw outlines of the general picture which develops as the laser-induced immune response. First of all, let us discuss the main question photobiologically—what is the primary chromophore, or photoacceptor, triggering the whole process?¹³ At present only one type of photoacceptor has been determined—dissolved molecular oxygen;¹⁴ however, other photoacceptors seem likely to act in other specific conditions.⁸

A photoexcited molecule—singlet oxygen—relaxes in microareas of water-based surroundings (blood, lymph, extra- and intracellular fluids) and acts in initiating the process of reconstruction of the metastable solution structure¹⁵ with the participation of hydrogen bonds.

Changes of physicochemical parameters of the solution cause a corresponding disturbance in the plasma membranes of the cells in the area,¹⁶ possibly, through conformational reconstructions of the integral membrane proteins connected to the water medium by their hydrate coating. It is important to underline that the reconstruction of bioliquids can be caused by other possible photoreceptors if their relaxation induces conformational transfer of biomacromolecules: the reconstruction of a hydrophilic protein area disturbs the hydrate coating which is likely to have a crystalline structure.³ The total effect is, without doubt, dose- and time-dependable.

Furthermore, nonspecific mechanisms have been identified which do not depend on the nature of the primary photoacceptors. We consider that at the cellular level membrane-modified effects develop first of all: namely, the activation of immunocompetent cells and possibly, changes in the antigenic parameters of tumoural cells, that could lead to the development of a photomediated autoimmune reaction of an organism against a tumour.³

We would like to underline as well that within the frames of the above developed conception it is possible to explain the changes discovered in a cytological structure of wound secretion after LLLT irradiation,¹⁷ as well as changes observed after the laser application for treating other diseases, such as angina pectoralis,¹⁸ nonspecific chronic pulmonary diseases¹⁹ and rheumatoid arthritis. If it is accepted that LLLT causes activation of the immune system, it seems to the authors entirely possible, even desirable, to use low reactive-level laser therapy (LLLT) for treating any form of immunodeficiency.

Conclusions

- (1) The most effective techniques with infrared LLLT in the preoperative period of oncological patients reduce the number of postoper-

ative complications, and do not lead to the increase in tumoural growth.

- (2) Immunological control data show the photostimulation of the immune system caused by laser irradiation.
- (3) LLLT induced in patients a cellular-humoral response of a nonthermal mechanism, without induction of any antigens.
- (4) A new form of the immune response is seen on the 4–5th day after the surgery.
- (5) The total intensity of the immune reaction in operated oncopatients *increases* during the period of control.

In this study, its results and discussion thereof, the authors have drawn only a general scheme of the possible laser stimulation of the immune system and tumoural antigen modification. It must be further checked qualitatively and showed to be formulated much more precisely, but the authors feel strongly that the findings are important enough to justify this preliminary report.

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