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The Use of the Infrared Laser Therapy of 890-910 NM for the Treatment Breast Cancer (Experimental and Clinical Study)

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Breast Cancer; 10 Years Results; Low Level Laser Therapy (LLLT); Experimental Tumors; Chemotherapy; Photodynamic Therapy

1. Abstract

The experimental studies were conducted with Walker's carcinosarcoma n256 (from the U.S.A. bank), cancer of the mammary gland (RMK-1). Spontaneous –mice with mammary glands cancer (type B). The tasks were as follows: to study the effect of different doses LLLT on the growth of experimental tumors. Evaluation of the effectiveness by applying LLLT in combination with various chemotherapeutic agents (vincristin, 5-Fu, ciclophosphan). Research of LLLR effect on different types of tumors show that this treatment can be used for tumors-statistic effect and for increasing life-span in animals. Applying LLLR in combination with chemotherapeutic agents causes tumors-static action. The most effective combination was LLLT + vincristin which caused the inhibition of tumor growth in Walker's carcinosarcoma-by 92,87%, in RMK-1 - by 90,29% in comparing to controls. The combination of LLLT with HpD showed - the effect of laser therapy takes place in 30 min before the irradiation and is accompanied by a sharp reduction of the preparation's accumulation in the tumor cells.

During research all patients were split into three groups. First group included 41 patients with breast cancer (II-III st.) that exposed to LLLT irradiation before surgery operation. Second group contain 38 patients with breast cancer (III-IV st.) that exposed to LLLT irradiation during postoperative period. Trird group contain 57 patients with breast cancer (IV st.) exposing LLLT irradiatin without surgery investigation. Observ of resaults stated decreasing of postoperative complication, stimulation of immune system, improving the quality of life and increasing the live-span. Using the most effective LLL treatment regiment, therapeutic pathomorphosis of the IIIrd degree was detected more than 50% tumor parenchyma disappeared due to necrotic and fibrotic tissues.

2. Introduction

Research into influence of LLLT on oncologic patients started in 1988 [1 ,2, 3]. The mechanisms and principles of using LLLT in this category of patients were studied (4,5,6,7,8,). The effect of LLLT on the immune system of cancer patients was investigated (2,9,10).

Now we have an experience of applicating LLLT on great amount of patients (more than 800) with the confirmed diagnosis of cancer at different stages (11). The main studies were conducted for cancer of the stomach (1,3,11, 12), colon (1,3, 11), rectum (1,3,11), esophagus (11, 13,14) and breast cancer (11,15,16,17, 18,19). The best results were obtained in the treatment of breast cancer.

Experimental studies were carried out on various types of breast cancer in vivo on mice and rats. The effect of different doses of LLLT on tumor growth was studied (20). We were looking for the most effective schemes of using LLLT with various groups of chemotherapy drugs (21,22,23,24). The possibility of using LLLT with photodynamic therapy was studied (25).

During clinical part of research all patients (136) were divided on 3 groups.

First group – LLLT was performed in patients with breast cancer before surgery – 41 patients (II-III st.).

Second group – LLLT was performed in patients with breast cancer in postoperative period- 38 patients (III-IV st.).

Third group – Patients (IV st) that were treated only by LLLT – 57.

Was observed decreasing postoperative complication, stimulation of immune system, improving the quality of life and increasing the live-span.

2.1. Background and Aims

1. Study influence of different doses of LLLT on the experimental tumor growth.
2. Study how laser radiation interacts with different groups of chemotherapy drugs and hematoporphyrins derivatives.
3. Apply the acquired knowledge to patients with breast cancer (II-III-IV st.) to evaluate the effectiveness of laser therapy.

2.2. Materials and Methods

Laser device - wavelength 890 nm, pulsed mode, pulse power 5W and 910nm, pulse mode and pulse power till 100W. Implanted tumors (Walker's carcinosarcoma, cancer of mammary glands (RMK-1) and spontaneous tumors (cancer of the breast in mice). Laser therapy influenced on patients breast cancer (II-III-IV st.) before and after surgery and as an independent method of treatment during symptomatic therapy. Histological investigations are carried out with tumor node and lymphatic node with staining haematoxylin and eosin. Blood of patients was assessed the state of immunocompetent cells, immunoglobulins, and tumor markers.

3. Results

3.1. Experimental Study: Investigations on the influence of different doses of lllt on the growth of experimental tumors

- **Aims of the Research**

The general strategy of our work lay in the following:

1. To study the effect of LLR on different types of implanted tumors (Walker's carcinosarcoma, cancer of mammary glands (RMK-1) and spontaneous tumors (cancer of the breast in mice).
2. To determine the most effective dosages of LLR affecting the growth of different tumors and prolonging the life-span in animals. (20) To study morphological changes in different types of exper¬imental tumors under the influence of LLR.

- **Materials and Methods**

In order better to verify the results of research we have done by that work on different types of tumors: Implanted Walker's carcinosarcoma (26 rats), implanted cancer of the mammary gland RMK-1 (75 rats) and spontaneous cancer of the mammary glands.

Walker's carcinosarcoma n256 (from the U.S.A. bank) implanted by a standard technique into female rats weighing 120-150 g. Laser irradiation added on the third day after the cancer implantation. Treatment lasted next 5 days. On the 7th day the animals were decapitated.

Cancer of the mammary gland (RMK-1) implanted by a standard technique into female rats weighing 85 g. All experimental animals were divided into 2 groups. First group:

Laser irradiation started on the 8th day after the inoculation. LLR lasted five days, and the animals were decapitated on the 13th day after the inoculation (30 animals). Second group:

Animals were decapitated on the 20th day after tumor implantation

After all, works and experiments we compared the number of animals in the control group to the number of survived animals in experimental groups.

All animals with implanted tumors were divided into three groups:

- TI group—the total dosage of irradiation was 0.46 J/cm2
- TII group—the total dosage of irradiation was 1.53 J/cm2
- TIII group—controls.

Walker's carcinosarcoma served for us as a training model because of its short period of duplication which is equal to a 2-3-day period. Due to this we have found out the most perspective treatment schemes of irradiation. RMK-1 having a slower growth process (the period of duplication is 4-6 days), served for us as a basic model on which we could check and prove the results obtained for LLR on Walker's carcinosarcoma.

Mice with spontaneous cancer of mammary glands (188 animals), type B, were divided into three groups according to the purposes of the research:

- Group Ml—to determine the most effective dosage of LLR for the life-span (45 animals).
- Group M2—to study histological changes under different dosages of LLR (60 animals).
- Group M3—to study the efficiency of LLR at different stages of tumors process (83 animals).

In groups Ml and M2 all animals had similar dimensions of tumors nodules 1.0 ± 0.3 cm and were subdivided into equal groups:

- Group 1A—LLR with the total dosage 0.03 J/cm2.
- Group 1B—LLR with the total dosage 0.3 J/cm2.
- Group 1C—controls.

Group Ml animals were observed for 30 days. Their life-span as well as the controls was determined and compared. In group M2 the animals were decapitated on the 8th day after the beginning of LLR treatment. In group M3, the animals received a total LLR dosage of 0.03 J/cm2. The group M3 animals were subdivided into equal groups according to the dimensions of tumor nodes:

- Group 3A—1.0 ± 0.3 cm (experimental—16, controls—17)
- Group 3B—2.0 ± 0.3 cm (experimental—17, controls—16).
- Group 3C—3.0 ± 0.3 cm (experimental—9, controls—8).

Laser therapy have been conducted during 8 days in all investigations. In this research used Ga-As semiconductor laser (wavelength 890 nm, pulsed mode, pulse power 5W). Histological investigations were carried out as follows: A thin plate cut out of a tumor node, then it was cut into several segments. This material has been putted into paraffin. Pieces as thick as 5-7 fim were stained with haematoxylin and eosin.

- **Results**

Results of treatment rats with Walker's carcinosarcoma on the 7th day after the inoculation and LLR we visually see more definite margins of the tumors node with the capsule formation (Figure 1). The weight of tumors was: Group TI (0.46 J/cm2) — 9.92 g ($p < 0.5$); Group TII (1.53 J/cm2) —11.58 g ($p > 0.5$); Group TIII (control) —11.28 g (Figure 2).

Thus, the increase in the dosage of 3.32 times did not produce tumor-static effect, and the TII group did not differ from the controls.

Histological investigations showed that histological changes do not depend on the LLLT dosage, and in the TI and TII groups. Changes in all groups were almost the similar; there were not any significant difference in their histological pictures. Both in the experimental.

Typical condition of rats with implanted Walker's carcinoma (n256) following laser therapy, on the 7th day post-implantation (Figure 1).

Weight of 7-day-old implanted Walker's carcinoma compared for laser therapy and control animals (Figure 2)

Histology of tumor from Walker's carcinoma implant showing changes after laser therapy (haematoxylin and eosin) (Figure 3).

While comparing the experimental and control groups one could see a tendency to the increased dystrophic changes in cells and larger areas of necrosis in the experimental groups. In those groups there were more pronounced impairments in microcirculation. Vessels of the capillary type were filled with erythrocytes; massive hemorrhagic foci were seen as well.

In group of rats with RMK-I after LLR tumor nodes did not differ visually from the controls (Figure 4).

Weight of 13-day-old implanted RMK-1 compared for laser therapy and control animals (Figure 5).

The difference between foci of necrosis, however in the tumor cells of the experimental group dystrophic changes were more pronounced.

Life-span of RMK-1-implanted rats compared for laser therapy and control animals

(Figure 6).

The difference between the rats which have been irradiated by the lower LLLT dose group and the other two groups was statistically significant ($p < 0.5$).

Research on mice showed that the life-span of animals treated with LLR was longer than in the control group of animals. In the TI group animals lived longer by 1.44 times, in the TII group 1.24 times respectively, in comparison to controls (Figure 7).

Histological analysis of tumors from the exper¬imental group showed more marked necrotic changes; two nodules were almost totally necrotized. However, in the preserved tumor parenchyma there was rather high mitotic activity (Figure 8). Significant difference between the histologic picture of the TI and TII groups was not observed.

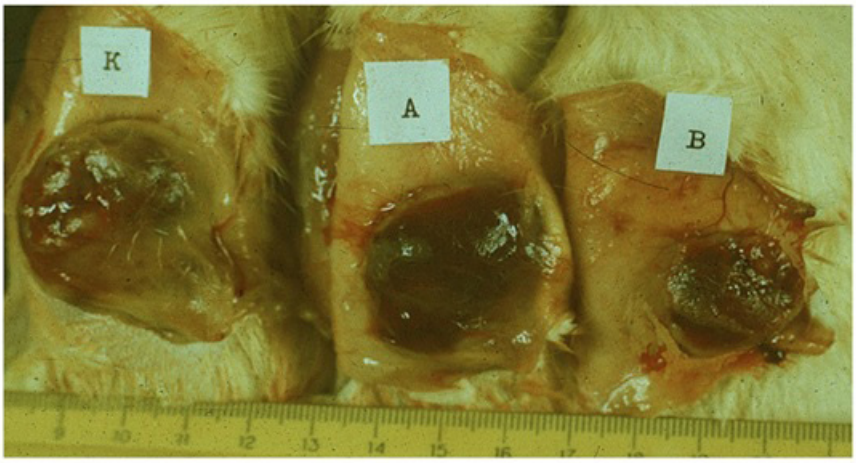

Figure 1: A—laser therapy, dose = 1.53 J/cm2; B—laser therapy, dose = 0.46 J/cm2; K—no laser therapy, control. Macroscopically, the tumor on the 0.46 J/cm2 irradiated animal is significantly smaller than the other two.

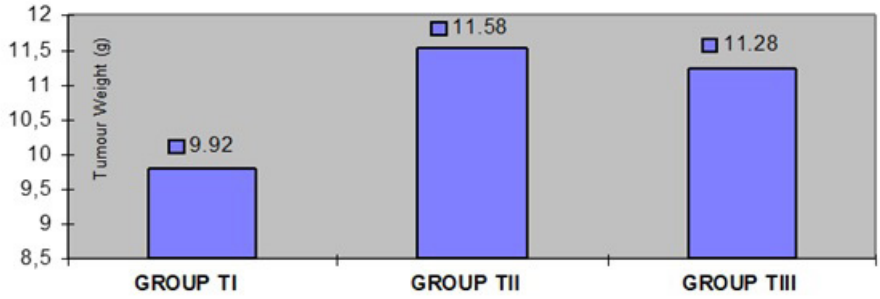

Figure 2: Group TI—0.46 J/cm2; group TII—1.53 J/cm2

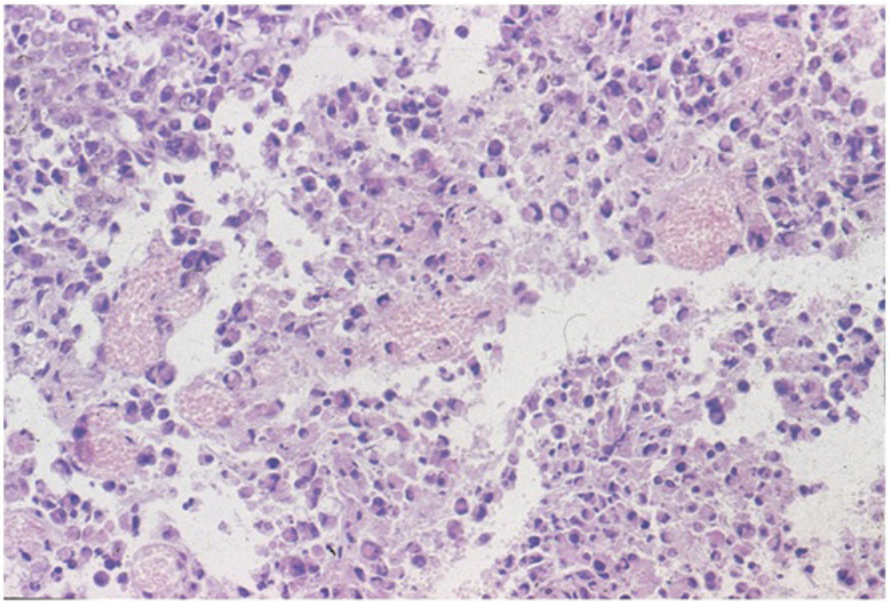

Figure 3: Marked dystrophic changes in the tumor cells can be seen accompanied by impairment to the tumor microcirculation groups and in the controls there were marked unspecific dystrophic changes, mostly, in the form of nucleic picnosis and cytoplasmic vacuolization. While comparing the experimental and control groups one could see a tendency to the increased dystrophic changes in cells and larger areas of necrosis in the experimental groups. In those groups there were more pronounced impairments in microcirculation. Vessels of the capillary type were filled with erythrocytes; massive hemorrhagic foci were seen as well.

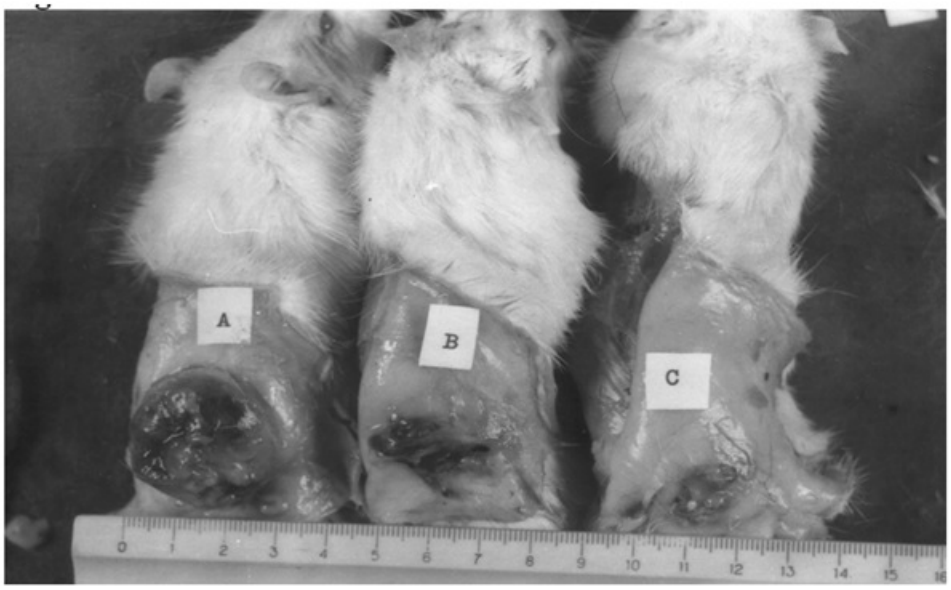

Figure 4: Typical representative condition of rats with implanted cancer of the mammary gland (RMK-1) on the 13th day post-implantation. C—laser therapy, dose = 1.53 J/cm2; B—laser therapy, dose = 0.46 J/cm2; A—no laser therapy, control. The tumor size in the rats treated with the lower LLLT dose is noticeably smaller than the others.

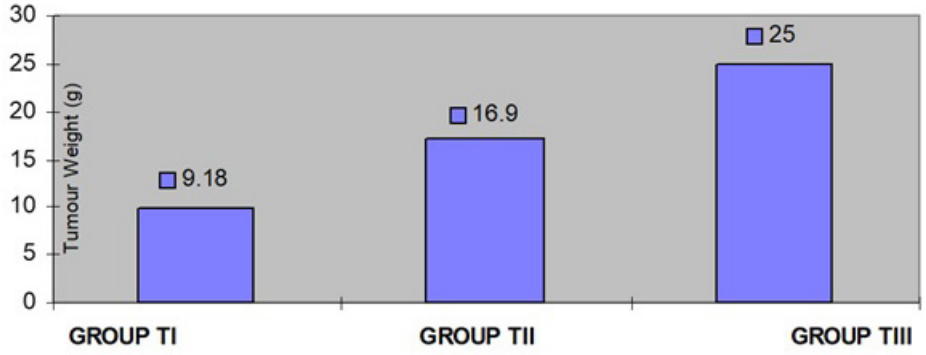

Figure 5: Weight of the RMK-1 tumor on the 13th day post-implantation compared for laser therapy and control animals. The difference between the rats in the lower LLLT dose group and the other two groups was statistically significant ($p < 0.5$). Group TI—0.46 J/cm2; group TII—1.53 J/cm2; group TIII—control, no irradiation.

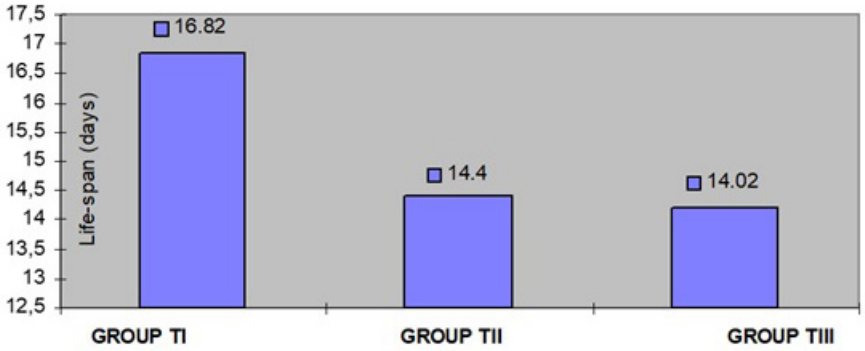

Figure 6: Life-span of RMK-1-implanted rats compared for laser therapy and control animals. Group TI—16, 82 days, group TII—14,4 days, group TIII—control -14,02 days.

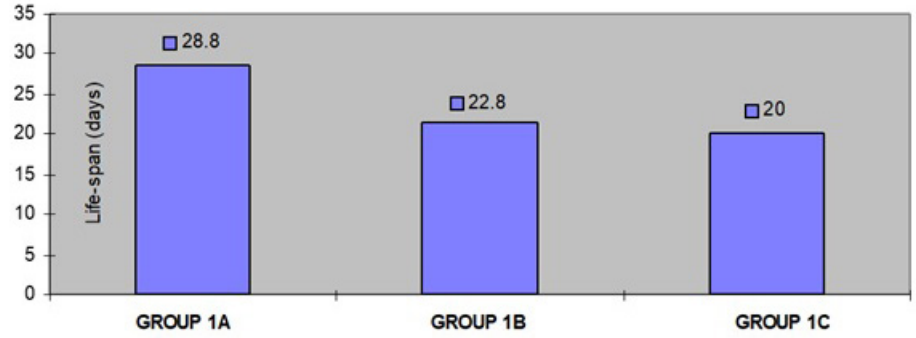

Figure 7: Group 1A—0.03 J/cm2; group 1B—0.3 J/cm2; group IC— control The difference between Group 1AI and the other two groups was statistically significant ($p < 0.5$).

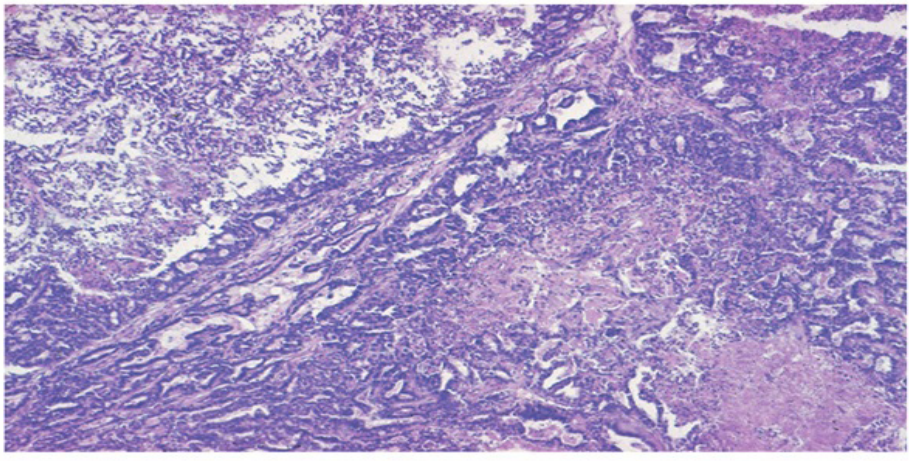

Figure 8: Typical example of histology of spontaneous cancer of the mammary gland in an LLLT irradiated mouse ((haematoxylin and eosin). Dystrophic changes are accompanied be foci of necrosis.

The effectiveness of laser therapy depended on the tumor dimensions. In group 3A life-span of animals with small tumors LLR increased by 1.53 times; in group 3B -test animals with larger tumor LLLT sowed lower efficacy—1.29 times in comparison to controls.

In group of mice with spontaneous mammary gland cancer (type A.B) we have studied only life-span of animals. The mice were divided into 3 groups - Group 1A—0.03 J/cm2; group 1B—0.3 J/cm2; group IC— control (Figure 7).

Researches showed that the life-span of animals treated with LLLT was longer than in the controls.

Histological analysis of tumors from the experimental group showed more marked necrotic changes two nodules were almost totally necrotized. However, in the preserved tumor parenchyma there was rather high mitotic activity (Figure 8)

- **Discussion**

Our research shows that LLR on different types of experimental tumors can produce different effects on their growth. Depending on the obtained dosage tumor growth can either be slowed down or be similar to that in the controls. It is worth noting that the growth of implanted tumors in rats (Walker's carcinosarcoma, RMK1) is slowed down at the dosage of 0.46 J/cm2 and also increasing dosage of up to 1.53 J/cm2 did not produce tumor-static effect. Tumors were insignificantly different by their weight and dimensions compared with the controls. The life-span of animals with RMK-1 was also longer by 1.2 times when the dosage was minimal. This tendency was proved during experiments on mice when the application of minimal dosages (0.03 J/ cm2) led to the increase of life-span by 1.44 times. Dosage increase in this case was also ineffective.

Histology analysis has shown that LLR increases dystrophic and necrotic changes in the tumor model. We showed also that morphologic changes were more pronounced in tumors with rapid growth (Walker's carcinosarcoma); while in tumors with slower growth and in more differentiated tumors these changes were less.

The efficacy of LLLT also depended on tumor dimension. The most effective application of LLR is observed at early stages of the disease when it can prolong the life of animals by 1.53 times. In more advanced tumor the efficacy of LLLT decreases.

- **Conclusions**

1. Investigations of LLR effect on different types of implanted and spontaneous tumors have shown that this treatment can be used for tumor-static effect and for increasing life-span in animals.
2. Different doses of LLR have different effects on tumors growth. The most effective dosages (in rats—0.56 J/cm2; in mice—0.03 J/cm2) lead to the retardation in tumors growth (in Walker's carcinosarcoma—by 12%; in RMK-1 in rats—by 63.8%). Life-span of animals was prolonged by 1.19 times in rats (RMK-1) and by 1.4 times in mice compared to the controls.
3. LLR efficacy also depends on tumor dimension. In animals with small nodules (1.0 ± 0.3 cm) LLR increased the life-span by 1.53 times. With the increase in tumors size the efficacy of LLR is decreased.
4. Histological analysis has shown that LLR increases dystrophic and necrotic changes in tumors. In rapidly growing tumors, (like Walker's carcinosarcoma) these changes are more pronounced than in slowly growing ones (RMK-1 in rats, spontaneous cancer of mammary glands in mice).

3.2. The Effect Of LLLT Combined With Chemotherapy On The Growth Of Experimental Tumors

Investigations on rats with an implanted- tumor carcinosarcoma of Walker's (31 animals), cancer of the mammary glands- RMK-1 (63 animals) - have shown that the application of low-level diode laser beam (890 nm) with some chemotherapeutic agents (vincristin,5-Pu,ciclophosphan) can affect the growth of experimental tumors (21,22,23,24). Laser therapy has been shown to be most effective before vincristin injection.

- **Aims of The Research**

The general strategy of our work lies in the following:

1. To study the effect of LLLR + chemotherapy on different types of implanted tumors (Walker's carcinosarcoma, RMK-1).
2. To determine the most effective dosages and combinations of LLLR + chemotherapy affecting the tumor growth.
3. To study morphological changes in experimental tumors under the LLLR + chemotherapy effect and chemotherapy alone.

- **Materials and Methods**

In order to varify obtained results of our research we have done second part of work on different implanted tumor types. Walker's carcinosarcoma has been implanted into 31 rats, cancer of mammary gland RMK-1 has been implanted into 63 rats accordingly. Accompanying chemotherapy included groups of chemotherapeutic agents on the fourth or sixth day after the implantation. The chemotherapeutic agents which used in therapeutic doses (Vincristin-4 mg/kg, 5-Ftoruracillin (5-Fu)-15 mg/kg, Cyolophospho-sphanum-80 mg/kg); this group of chemotherapeutic agents have been chosen because of it's ubiquitous used in chemotherapy. Walker's carcinosarcoma N 256 (from the U.S.A. bank) was implanted by a standard technique into female rats (each weight 120-150 g). Chemotherapy was performed on the fourth day after the implantation. On the 7th day the animals were decapitated.

All animals were divided into four groups:

T_I group – treating animals only with chemotherapeutic agents.

T_{II} group – treating animals with LLLR session dosage 0.6 J/cm2, 30 min, before the infusion of chemotherapeutic agents.

T_{III} group – influence on organism by chemotherapeutic agents after LLLR, first – 30 min after the infusion, second time- 24 hour after the infusion; the total dosage of irradiation was 0,12 J/cm2.

T_{IV} group – controls.

The TI-TIII groups were divided into three parts. Each part exposed the infusion of certain chemotherapeutic agents. Cancer of the mammary gland (RMK-1) was implanted by a standard technique into female rats (each weighing 85 g). Chemotherapy added on the sixth day after the implantations. On the13-th day the animals were decapitated.

All animals with tumors were divided into four group and every group was divided into three subgroups depending on the variety of chemotherapeutic agents:

M_I group – influence by only chemotherapeutic agents,

M_{II} group – influence by LLLR 30 min before the infusion of chemotherapeutic agents,

M_{III} group – influence by LLLR in 30 min after the infusion of chemotherapeutic agents,

M_{IV} group – controls.

The MI-MIII groups were divided into two parts. First part was subjected to be influenced by LLLR using dosage 0,6 J/cm2. Second part was subjected to be influenced by LLLR using dosage 0,06 J/cm2. Walker's carcinosarcoma served as a training model because of its short period of duplication within 2-3 days period. Due to this we have found out the most perspective schemes of LLLR with chemotherapeutic agents. RMK-1 having a slower growth process (the period of duplication is 4-6 days), served for us a basic model on which we could check and prove the results obtained for LLLR + chemotherapy on Walker's carcinocarcoma. Besides we continue to examine the effect of different doses of laser therapy application with GaAs semiconductor laser (wavelength 890nm., pulsed mode, pulse power 5 W.). Histological investigation was carried out on tumor node, then it was out into several segments. This material was put in paraffin. Pieces as thick as 5-7 ^Lm were stained with hematoxylin and eosin.

- **Results**

The results show that influence on cancer tissue by LLLR has the most effective results before adding chemotherapy, as well as the most effective chemotherapeutic drug is vincristin (Figure 9).

Walker's carcinosarcoma in rats after the inoculation and chemotherapy with LLLR the weight of tumors was (Table 1).

The most effective one was was vincristine, under influence of that drug the weight of tumors was 76.24% lower than in the control group (statistically significant ($p < 0.5$).

The graph presented helps to analyze results more effectively

The weight of tumor nodes in rats with Walker's carcinosarcoma on the 7th day after chemotherapy (gr.) (Figure 10).

Histological investigation has shown that the signs of therapeutic pathomorphosis were more marked under LLLR before chemotherapy than under simple chemotherapy. Under LLLR before chemotherapy there were established the microcirculatory disturbances and dystrophic changes in tumor cells.

The influence on RMK-1 in rats with LLLR before chemotherapy technique was more effective, than with inverse technique – chemotherapy before LLLR. Vincristin was the most affective agent than other ones (Figure 11).

The weight of tumor nodes in rats with RMK-1 on the13 day after the implantation and on the 7th day after chemotherapy was as follows (Table 2).

The graph presents result more effectively (Figure 12).

In this variety of tumors histological changes after LLLR + chemotherapy were more marked dystrophic changes in tumors cells under chemotherapy alone.

Table 1: The weight of tumor nodes in rats with Walker's carcinosarcoma on the 7th day after chemotherapy (gr.)

Chemotherapeutic agents	Groups			
	T_I	T_{II}	T_{III}	T_{IV}
5-Fu :2,5	2+0,79	4+0,2	1,93+0,73	8,42+3,3
Cyclophosphanum	3,52+1,38	2,20+1,04	3,32+1,03	
Vincristin	1,96+0,62	0,6+0,23	1,60+0,5	

Table 2: The weight of tumor nodes in rats with RMK-1 on the13 day after the implantation and on the 7th day after chemotherapy (gr.).

Chemotherapeutic agents	Groups			
	T_I	T_{II}	T_{III}	T_{IV}
5-Fu :2,5	2,98+1,54	1,05+0,43* 0,73+0,11	2,05+1,0* 1,48+0,72	5,67+0,87
Cyclophosphanum	2,62+1,24	1,65+0,6* 0,70+0,36	2,50+1,0*	
Vincristin	1,95+1,0	0,90+0,2* 0,55+),15	1,10+0,8* 1,1+0,5	

* - dosage 0,06 J/cm2

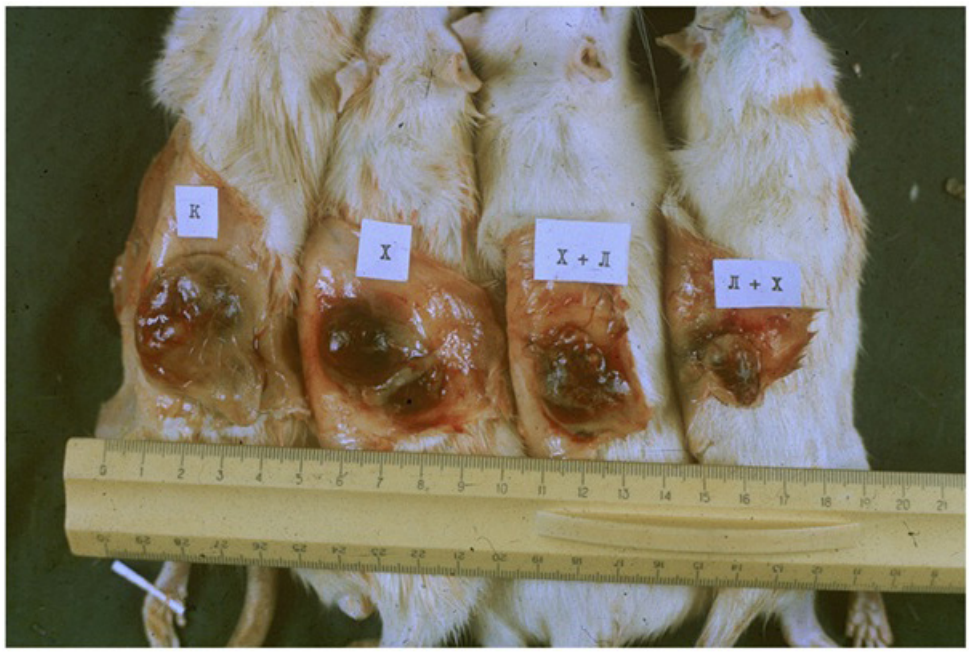

Figure 9: K – controls, X - vinctistin, X+JI - infusion of vicristin, followed by twice the LLLT - first - 30 min after the infusion, second - 24 hour after the infusion((total dosage 1,2 J/cm2) JI+X - single application of LLLT (dosage 0.06 J/cm2) for 30 minutes before the administration of the chemotherapeutic agent.

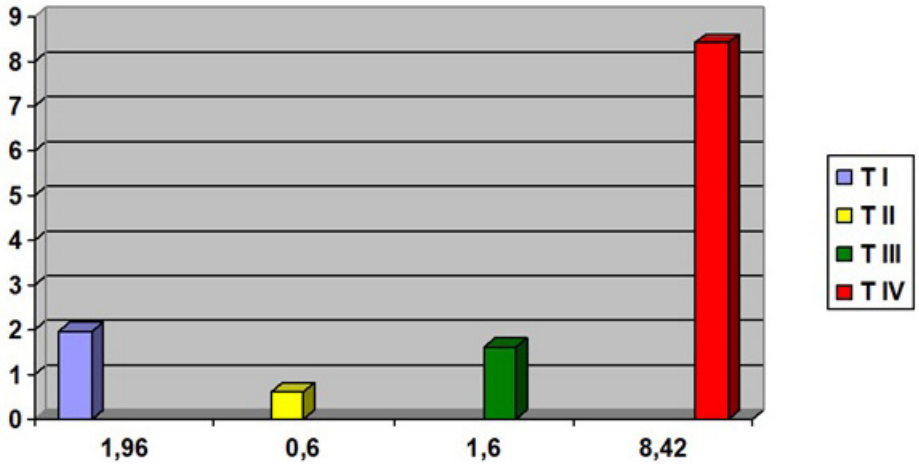

Figure 10: TI – only chemotherapeutic agents. TII - single LLLT session, 30 min, before the infusion of chemotherapeutic agents (.dosage 0.6 J/cm2). TIII - infusion of chemotherapeutic agents after LLLT, first - 30 min after the infusion, second time- in 24 hour after the infusion; t (total dosage -0,12 J/cm2). TIV - controls.

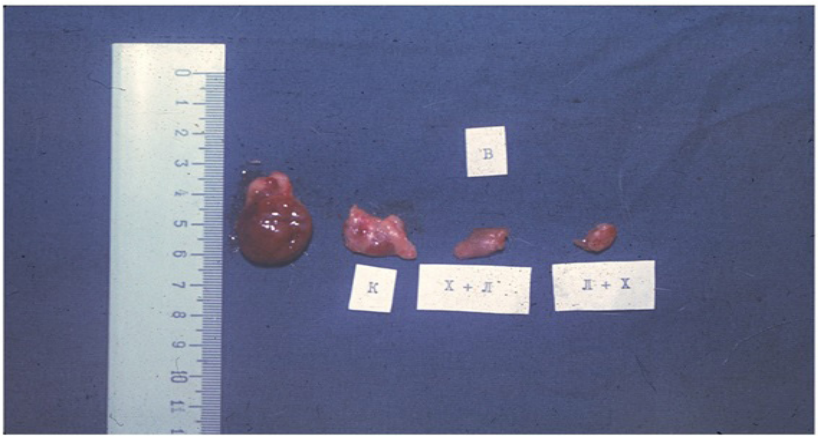

Figure 11: K – controls, X - vinctistin, X+JI - infusion of vicristin, followed by twice the LLLT - first - 30 min after the infusion, second - 24 hour after the infusion((total dosage 1,2 J/cm2) JI+X - single application of LLLT (dosage 0.06 J/cm2) for 30 minutes before the administration of the chemotherapeutic agent.

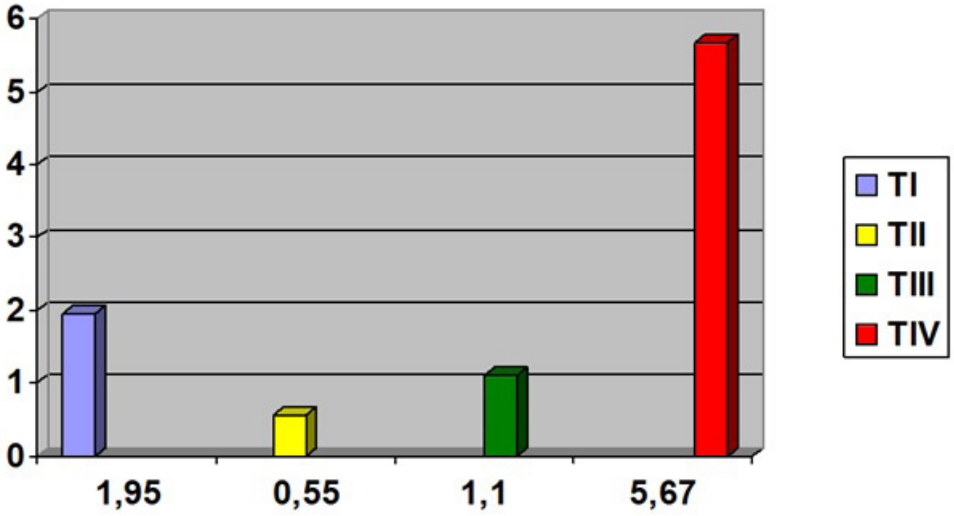

Figure 12: TI – only chemotherapeutic agents. TII - single LLLT session, 30 min, before the infusion of chemotherapeutic agents (dosage 0.6 J/cm2). TIII - infusion of chemotherapeutic agents after LLLT, first - 30 min after the infusion, second time- in 24 hour after the infusion; t (total dosage -0,12 J/cm2). TIV - controls.

Fluorescent intensity in relative units

- **Discussion**

Our research show that LLLR has the most effective results when it is applied before the injection of chemotherapy. While in rats with Walker's carcinosarcoma injectionised by only one chemotherapic drug that leaded to the inhibition in tumor growth by 60,4% in average. In comparison to controls; LLLR in the dosage 0,6 J/cm2 before the injection of chemotherapy lead to tumor growth inhibition by 83,3%

The combination of chemotherapy before LLLT was noted less effective - tumor growth was retarded by 73,27% in comparison to controls.

In rats with RMK-1 application of LLLR in the same dosage (0,6 J/cm2) before the chemotherapy injection lead to the tumor growth inhibition by 88,35%, in average; the combination of chemotherapy before LLLT was less effective and inhibited the growth by 71,43%, in average, in comparison to controls. Then, LLLT before the injection of chemotherapy, to our opinion, stimulates the immune system of animals more effectively; it reliably reduced toxic effect of chemotherapy and increased the efficiency of the treatment. Less efficiency of LLLT (0,6 J/cm2) applied after the injection of chemotherapy can be explained by the fact that the inhibition of the immune system has already developed, the organism moved, to the lower level of excitation and the stimulation of the immune system of an animal was not considerable. The fact that the reduction of LLLT dose in 10 times (up to 0,06 J/cm2) sharply reduced the efficiency of LLLT with chemotherapy supports the hypothesis that this efficiency depends on the immune system status, i.e. this dosage was little effective for achieving your immuno-stimulating action. Histological studies also proved this fact; they have shown that there were no statistically significant changes in the histological picture of tumors after LLLR with chemotherapy and in tumors which were treated only with chemotherapy. Further investigations (22) have proved that the therapy efficiency also depends on LLLR dosage and scheme. If it important to underline that chemotherapy drugs different by their mechanism of action produce various effects in combination with the same dosages and. regimes of LLLR treatment. The most effective was combinations with vincristin: in rats with Walker's carcinosarcoma the tumor growth was inhibited by 92,87% while in animals with RMK-1 by 90,29%. Taking into account the fact that tumors static mechanism of vincristin is based on its impact on protein compounds of DMA and mitotic processes in a cell, we cannot exclude the increase of the effect of this preparation and laser beam on a cellular level.

- **Conclusions**

1. Studies of the LLLR effect on inoculated tumors of various types have shown that LLLR can be used in combination with chemotherapeutic agents for achieving tumors-static action.
2. Various schemes of LLLR application in combination with chemo-preparations also produce various effect on the tumor growth. The most effective was LLLR application (0,6 J/cm2) before the injection of chemotherapy drugs; this scheme causes the retardation in tumor growth: in Walker's carcinosarcoma – by 83, 3%in average; in RMK-1 – by 88,3% in average, comparing to controls.
3. Decrease of LLLR dosage up to 0,06 J/cm2 (RMK-1) reduced the efficiency of LLR + chemotherapy.
4. The most effective was the scheme LLLR before vincristin application which caused the inhibition of tumor

growth in Walker's carcinosarcoma-by 92,87%, in RMK-1 - by 90,29% in comparing to controls,

5. Histological studies have shown the absence of considerable difference between LLLR + chemotherapy and chemotherapy without supplying LLLR.

3.3. Study of the accumulative function of tumor cells after the injection of hematoporphyrine derivatives (HpD) under the irradiation with LLLT.

The possibility of using LLLT with photodynamic therapy was studied on Walker' s carcinosarcoma N 256 from USA bank (25).

The selectivity of accumulation or irradiation of different chemotherapeutic drugs in tumor tissue is one of the main factors determining their curative-diagnostic efficiency, Pharmacokinetics of hematoporphyrine derivatives (HpD) in intact and diseased organism has been studied by some researchers (26,27,28,29), However, changes in HpD pharmacokinetics under some side-effects (for example, the tumor process at different stages, the inflammatory process in the organism, etc.) have not been studied in fact. The tumor specificity of these preparations depends on the chemical structure, changes in their transport and in the organism damaged by the disease. Various preparations are "bound in a different way by normal and tumor cells. We preferred HpD synthesized in USSR as it has the greatest possibility to accumulate in tumor cells (26).

- **Aims of the Research**

The general strategy of this work lies in the following:

1. To study the accumulation of HpD in the tumor under different schemes of LLLT and to determine the best regime which effects the accumulation most strong.
2. To determine terms of significant changes in HpD accumulation and discharge from the tumor tissue under LLLT.

- **Materials and Methods**

The experiments have been done on white female rats weighing 120-150 gr divided in 4 groups, 47 animals in each, Walker' s carcinosarcoma N 256 from USA bank was inoculated subcutaneously in the left lateral part of the body in dosage 0,5 ml. The peculiarity of this tumor lies in a rapid growth with the period of duplication in 2-3 days. The experimentation was done on the 4-5th/ day after innoculation when possible spontaneous necrosis and peripheric hemorrhages are absent. The preparation, a derivative of hematoporphyrine (HpD) synthesized in Institute of Chemical Technology (prof, Mironov A.) was injected in the dosage 10 mg/ kg under the ether narcosis intravenously in the jaguar vena. The accumulation of HpD in the tumor was stu¬died in each animal decapitated in 0,5, 3, 6, 12, 24 and 48 hours. Cryostatic spieces 18-20 mkm. thick were prepared from the tumor; then they were studied with microspectro-flowmetric methods, (a fluorescent microscope "LOMO", a meter from "OPTON"), HpD fluorescence was exited by the light with = 400 nm, a probe N 14 with = 624 nm. The fluorescence intensity was measured qualitatively without the fluorescence background and was calculated in relative units. laser irradiation was performed by a semiconductor laser "UZOR" (Ga-As) with the wavelength 890 nm. and the pulse power 5 wt.

- **Results**

LLLT was applied one time as well as many times; and it was done both before HpD injection and after it. Each series of animals has been divided into two groups;

they got different dosages of irradiation - minimal (0,03067 G/ cm2) and maximal (0,3067 G/cm2). In the first group animals were injected only with HpD. They were controls. In the second series animals were irra¬diated once immediately after HpD injection. In the third series animals were irradiated twice: first time -immediately after HpD injection, second time - in 30 min after the first one. In the fourth series animals were irradiated once 30 min before HpD injection. In the fifth series animals were irradiated twice: the first time -1 day before HpD injection, the second time - 30 min before HpD injection (Figure 13).

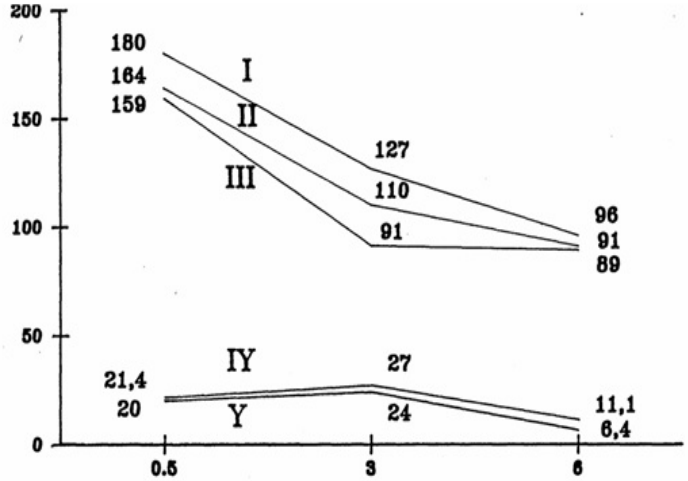

Figure 13: I. Injection of only HpD (controls). II. Direct single (max. dosage) irradiation after HpD injection. III. Double irradiation (minimal dosage) immediately and 30 min after HpD injection. IV. Single irradiation (max. dosage) 30 min before HpD injection. V. Double irradiation (minimal dosage) 1 day and 30 min before HpD injection.

In the second and third series the accumulation of the preparation in the tumor practically does not differ of that in controls. In the third series the accumulation was somewhat lower than in the controls; however, the difference was not statistically significant. Different dosages of irradiation practically did not inf¬luence the preparation's accumulation. In the fourth and fifth series a significant reduction in this accumulation in the tumor has been found out, and this phenomenon has been watched in all periods of investigation and the fluorescence intensity was almost similar. In these series of investigations the quantity of the absorbed sensitizer depended little on the dosage of irradiation, How eve, in the fifth and fourth series it was found out that under the minimal dosage of irradiation (0,03067 G/cm) the photo- sensitizer was accumulated less than under the maximal one, The most interesting results are presented in Table 3.

The graph presented helps to analyze results more effectively. Accumulation of hematoporphyrin derivative (HpD) in the tumor tissue of experimental animals using different parameters of laser therapy (Figure 13).

Table 3: A statistical analysis of the data obtained revealed the following results expressed in relative units.

Time after HpD	1 ser,	2 ser,	3 ser.	4 ser,	5 ser.
30 min	180±15,5	164±18.5	159±13.6	21,4±6,2	20±6,2
3 hours	127±8,8	110±I4,1	99,8±14,3	27,2±2,3	24,4±4.8
6 hours	96,4±17,0	91±19,3	89±8,5	11,8±2,3	6.4±2.1

- **Discussion**

This research has been made by us on the base of our data obtained for the analysis of LLR effect on inoculated tumors.

At first, taking into consideration the research made by T. Ohshiro (30) we expected that LLR would increase the accumulation of HpD and thus, it could, be used for the improvement of photodynamic therapy effect. According to the theory LLR had to effect the tumor blood circulation through changes in the vascular wall and cellular membranes permeability. However, in the II and III series of investigations when LLR was used after HpD injection were did not observe any significant changes in the preparation accumulation and discharge comparing to the controls. We suggested that those changes which had to take place after LLR had not developed yet. In the fourth series when LLR was made once before HpD Injection the insignificant reduc¬tion of the preparation accumulation in the tumor has been noted. In the fifth series when LLR was made 24 hours before HpD injection a sharp reduction in the quantity of photosensitizer in the tissue was found out; more over during all periods of the investigation it was similar **(Figure 13)**. The preparation accumulation depended little on the irradi¬ation dosage. However, in the fourth and fifth series under minimal dosages the preparation accumulated less than under maximal ones.

Thus, the investigation made presented a clear picture of HpD accumulation in tumor tissue. The effect of laser therapy takes place 30 min after beginning and is characterized by a sharp reduction of the preparation in tumor tissue. It could be explained both by the impairments in the preparation's transport through the vascular floe (to be more exact, through the capillary wall) and by the impairments of that transport through the cellular membrane.

The reduction of the preparation's discharge out of cells irradiated by laser in comparison to controls also supports this suggestion.

- **Conclusions**

1. Laser irradiation is the most effective when being performed 30 min before the HpD injection. HpD accumulation in tumor tissues is reduced by 111% in comparison to controls
2. Changes in the accumulation of HpD and disturbances in its release when using laser therapy are caused by changes in permeability both in the capillary wall and in the cell membrane.
3. Laser therapy after HpD injection does not influence the preparation accumulation in tumor tissues.

4. Clinical Study

4.1. Results of treatment in patients with Breast cancer ((II, III, IV st.) treated by LLLT and combination of LLLT and surgery (10-year experience).

- **Aims of the Research**

The general strategy of this work was the following:

1. To study how the use of LLLT affects the blood and immune status of patients and determine the duration of the effect of LLLT at different stages of the disease.
2. To determine how the use of LLLT can affect the quality of life in patients with stage IV of disease.
3. How the use of LLLT influence on the efficiency of surgery in patients with IIa-IIIa st.
4. To study what histological changes occur in tumor tissue after using LT.
5. Compare the life expectancy of patients (II-III st.) with surgical treatment and patients who received LLLT before and after surgery.

- **Materials and Methods**

All patients were divided into 3 groups:

1group - Only LLLT- in 57 patients (IV st.).

2 group - After combined treatment LLLT was used in 38 patients (III st.).

3 group - With surgery LLLT was used in 41 patients (II-III st.).

Was used Ga As laser with frequency up to 3 000 Hz, power up to 5 Wt.

We used some program of LLLT:

1. Programs to reduce pain (5 days)- irradiation on projection of tumor and metastasis - with the dosage 1,53 J /cm2 (1 group)

2. Programs for immune stimulation (5 days) - irradiation on projection of immuno-producing organs with the total dosage 0,49 J / cm2 (1,2 group).

3. Programs using LLLT before surgery (5days) -)- irradiation on projection of tumor and lymphatic nodes with irradiation of projections of immuno-producing organs (3 group).

4. Programs using LLLT after surgery (2days) – irradiation postoperative suture and area of lymphatic nodes (3 group).

Analyses were made 1-2 days before laser rtherapy,1-2 days after laser therapy and 4-5 days after surgery.

Comparative white blood count (leucocytes, lymphocytes, monocytes) was carried out by the method of direct calculation 'under a microscope in Gorjaev's chamber. Immunoglobulin components (IgA, IgM, IgG) in blood serum were determined on the Ps-9 (Labsystems) T-lymphocytes were assayed according to Hasnill. The determination of T-active rosette-formed lymphocytes (Trf), T-helpers (Th), T-suppressors (Ts) was performed by a standard technique (6).

Histological investigations have been conducted Prof. Frank G.A. and Prof Voltchenko N.N.

Histological changes were divided into 4 degrees:

I degree - tumor parenchyma is alive, dystrophic changes in tumors are seen.

II degree - the lager part of tumor parenchyma is alive, but market necrotic and fibrotic foci were observed.

III degree - more then 50% of tumors parenchyma disappeared due to necrotic and fibrotic foci.

IV degree - total tumor resorbtion.

4.2. Results of using LLLT in patients with IV st. of Breast cancer (57 patients)

In this part of our study, we wanted to find out how the use of LLLT affects the hematological and immunological status of patients and how LLLT affects the quality of life of this category of patients. These patients received LLLT when for one or another reason combined treatment was not carried out or after the combined treatment with the progression of the process.

All patients were separated on 3 groups:

I group – patients with pain syndrome - metastasis in bones, sternum, spinal cord (18 patients).

II group- patients with loco-regional metastasis (19 patients).

III group- patients with loco-regional and remote metastasis (20 patients).

On age structure the patients of skilled groups were distributed as follows (Table 4).

According to histology diagnosis patients were distributed as follows (Table 5).

After statistical processing dynamics of crates of "white" blood looked as follows (Table 6).

Taking into account, that the definition of immunoglobulins was defined in various laboratories on various techniques, we bring standard units of the rather normal contents of immunoglobulins (Table 7).

Dynamics of fractions of T-lymphocytes of the patients with Breast cancer IV stage received LLLT looked as follows (Table 8).

The statistical processing of the received results has shown reliability of changes on the basic parameters, except for parameters marked - *. Changes of these parameters more evidently can be expressed graphically.

Changes in various blood parameters were most significant in the II group Leucocytes, lymphocytes and monocytes reacted on laser therapy their increasing.

Dynamics Ig A has shown, that laser therapy increased their number in 4,84 times. Ig M reacted on laser therapy in 9,79 time their increasing. Ig G increased in 5 time.

In I and III groups these changes were not so reliable.

Changes of active T- lymphocytes was characterized by their increase in II and III groups.

It was found reliable increasing T rf. and T help. The ratio of T supr. was decreased significantly.

A survey of patients of all groups showed that the most reliable improvement in well-being can be noted in 17% of patients, reduction of pain syndrome in 12% of patients, normalization of sleep in 7%, improvement of appetite in 2% of patients. At the same time, 38% of patients had a change in their state of health, the remaining 62% of patients did not notice a change in their state of health.

Table 4: Distribution of the patients with Breast cancer IV stage by age groups.

Age	up to 40	41-50	51-60	61-70	71-80	81-90
I group	2 11,11%	6 33, 33%	5 27,77%	3 16,66%	2 11,11%	
IIgroup		5 26,31%	7 36,84%	3 15,8%	3 15,8%	1 5,26%
III group	1 5,0%	6 30,0%	5 25,0%	4 20,0%	4 20,0%	

Table 5: Histological diagnosis of the patients with Breast cancer IV stage received LLLT

Histological diagnosis	I group	II group	III group
Invasive ductal carcinoma (NST)	9(7*)	9	9 (9*)
Invasive lobular carcinoma	3(2*)	4	5 (5*)
Pure tubular carcinoma	2(2*)	4	2 (2*)
Mucinous carcinoma	1(1*)		1 (1*)
Medullary carcinoma		-	1 (1*)
Invasive cribriform carcinoma		1	1 (1*)
Invasive papillary carcinoma	1(1*)	1	1 (1*)
Paget's Cancer	1		

* -histological verification of the remote metastasis

Table 6: Dynamics of crates of «white» blood cells of the patients with Breast cancer IV stage received LLLT (1x109).

		I group	II group	III group
Leukocytes	Before LLLT	9,9±2,58	8,7±1,58	9,2±3,4
	After LLLT	9,4±2,99*	9,1±2,17	8,23±2,61*
Lymphocytes	Before LLLT	2,55±1,13	2,31±1,13	2,63±1,98
	After LLLT	2,23±1,58*	2,49±0,52	2,51±0,69*
Monocytes	Before LLLT	0,48±0,34	0,27±0,14	0,50±0,43
	After LLLT	0,52±0,11*	0,31±0,89	0,46±0,22*
Granulocytes	Before LLLT	5,58±1,24	6,21±2,32	6,34±2,51
	After LLLT	5,77±1,43*	6,34±1,84*	6,41+1,97*

* - p> 0,5 (Doubtfully).

Table 7: Dynamics of crates of immunoglobulins of the patients with Breast cancer IV stage received LLLT (standard units).

		I group	II group	III group
Ig A	Before LLLT	+ 9,29	+21±1,58	+11,26
	After LLLT	+9,4 *	+101,59	+9,94
Ig M	Before LLLT	+2,41	+1,48	norm
	After LLLT	+ 3,12	+14,53	+9,40
Ig G	Before LLLT	+2,48	norm	norm
	After LLLT	+4,89±0,11*	+5 ±0,89	+7±0,84*

* - p> 0,5 (Doubtfully).

Table 8: Dynamics of fractions of T-lymphocytes of the patients with Breast cancer IV stage received LLLT (standard units)

		I group	II group	III group
T rf.	Before LLLT	530±91,8	485±41,1	450±88,6
	After LLLT	500±156,1	510±74,2	490±63,76
T help	Before LLLT	210±21,3	160±88,5	180±61,4
	After LLLT	205±57,9	240±54,1	290±110,3
T supr	Before LLLT	290±99,3	255,0±93,8	275,0±33,5
	After LLLT	275±35,1*	230±73,27	240±70,42

* - p> 0,5 (Doubtfully).

4.3. Results of using LLLT in patients with III st. of Breast cancer (38 patients)

In this part of our study, we wanted to find out how patients with a long period of remission respond to laser therapy. We studied the dynamics of blood parameters, immunoglobulins and various classes of T- lymphocytes.

On age structure the patients of skilled groups were distributed as follows (Table 9).

As it is visible from the table the essential distinctions in age structure in all groups were not.

According to histology diagnosis the patients were distributed as follows (Table 10).

After statistical processing dynamics of crates of "white" blood looked as follows (Table 11).

Taking into account, that the definition of immunoglobulins was defined in various laboratories on various techniques, we bring

standard units of the rather normal contents of immunoglobulins.

(Table 12).

Dynamics of fractions of T-lymphocytes of the patients with Breast cancer III st. received LLLT looked as follows (Table 13).

The statistical processing of the received results has shown reliability of changes on the basic parameters, except for parameters marked - *.

Dynamics of the immunoglobulins at the patients, which received laser therapy marked the following tendency: Dynamics of Ig A has shown, that the laser therapy influence their quantity: prior to the beginning use of LLLT — +10±7,21, after the ending of LLLT – +46,8,1. Laser therapy increased the amount Ig A more than 4 times.

Ig M changed with - +2,34 up to + 7±1,61 after laser therapy. Laser therapy increased the number Ig M more than 10 times.

The initial parameters Ig G increased after laser therapy from norm, till 7±0,23.

Thus, laser therapy significantly increased the amount Ig A and IgM. The changes Ig G were unreliable.

Changes of active T- lymphocytes (Trf.) was characterized by their increase with 500±95,7 up to 540±159,8 after laser therapy.

The change of T-help was characterized by their increase with 175±55,3 up to 240±11,9 after laser therapy.

The quantity of T-supr. decreased from 250±21 till 235±87,79 after laser therapy. But these changes were unreliable.

Studies have shown, that patients with III st. of Breast cancer (remission stage) react to laser therapy by increasing the number of lymphocytes, Ig A and Ig M and increasing the number of active T-lymphocytes, T help. and T supr.

Table 9: Distribution of the patients with Breast cancer III stage by age groups

Age	up to 40	41-50	51-60	61-70	71-80	81-90
Number of patients	2 5, 26%	10 26, 31%	14 36,84%	6 15,79%	5 13,15%	1 2,63%

Table 10: Histological diagnosis of the patients with Breast cancer III stage received LLLT

Histodiagnosis	Number of patients
Invasive ductal carcinoma (NST)	18
Invasive lobular carcinoma	11
Pure tubular carcinoma	5
Mucinous carcinoma	1
Medullary carcinoma	2
Invasive cribriform carcinoma	1
Invasive papillary carcinoma	
Paget's Cancer	

Table 11: Dynamics of crates of «white» blood cells of the patients with Breast cancer III stage received LLLT (1x109)

Leukocytes	Before LLLT	7,4±1,12
	After LLLT	9,5±1,84
Lymphocytes	Before LLLT	2,07±0,98
	After LLLT	2,42±1,34
Monocytes	Before LLLT	0,27±0,08
	After LLLT	0,29±1,53*
Granulocytes	Before LLLT	5,61±1,95
	After LLLT	6,47±1,16

*- p> 0,5 (Doubtfully).

Table 12: Dynamics of crates of immunoglobulins of the patients with Breast cancer III stage received LLLT (standard units).

Ig A	Before LLLT	+10±7,21
	After LLLT	+46,81
Ig M	Before LLLT	+2,34
	After LLLT	+25,11
Ig G	Before LLLT	norm
	After LLLT	+7 ±0,23*

* - p> 0,5 (Doubtfully).

Table 13: Dynamics of fractions of T-lymphocytes of the patients with Breast cancer IIIstage received LLLT (standard units)

T rf.	Before LLLT	500±95,7
	After LLLT	540±15,8
T help	Before LLLT	175±55,3
	After LLLT	240±11,9
T supr	Before LLLT	250±21,5
	After LLLT	235±87,79*

* - p> 0,5 (Doubtfully).

4.4. Results of treatment in patients with IIa-IIIa st. Breast cancer treated by combination of LLLT and surgery (10-year experience).

- **Materials and Methods**

LLLT was performed in 41 patients with conformed diagnosis of breast cancer IIa -IIIa st., control group had 40 patients.

Distribution of age was from 37-76 years and in the experimental and controls group was more or less similar.

Used Ga As laser with frequency up to 3 000 Hz, power up to 5 Wt.

Before surgery LLLT was used during 5 days

After surgery LLLT was used on all patients during the 2 days from the start of treating. Postoperative suture was irradiation in total dosage 0,041 J/cm2. Area, of lymphatic nodes was irradiation with the total dosage 0,006 J/cm2.

Later LLLT was used in 3,6,12,18,24 months during 5 days each time like it was done in the I group of patients.

Comparative white blood count (leucocytes, lymphocytes, monocytes) 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) T-lymphocytes were assayed according to Hasnill.

The determination of T-active rosette-formed lymphocytes (Trf), T-helpers (Th), T-suppressors (Ts) was performed by a standard technique.

Analyses were made 1-2 days before laser therapy, 1-2 days after the therapy and on the 4-5 day after surgery.

All Patients were divided into 3 Groups:

I group – patients with irradiations of projections of organs associated with the immune system with the total dosage 0,49 J/ cm2 (4 patients).

II group – patients with irradiations of projections of organs associated with the immune system with the total dosage 0,49 J/cm2 and irradiation of tumor projections and lymphatic nodes with the dosage 0,24-0,46 J/ cm2 (4 patients).

III group – patients with irradiations of organs associated with the immune system with the total dosage 0,49 J/ cm2 and irradiations of the tumor projections and lymphatic nodes with the dosage 0,46 -1,53 J/ cm2 (33 patients). In this group LLLT was used after surgery on the 1 day (duration 2 days). Postoperative suture was irradiation in total dosage 0,041J/cm2

Area of lymphatic nodes was irradiation with total dosage 0,006 J/ cm2

Histologic changes were divided into 4 degrees:

I degree – parenchyma of the tumor is alive, degenerative changes in tumor parenchyma are seen.

II degree – the larger part of tumor parenchyma is alive, but marked necrotic and fibrotic foci were observed.

III degree – more than 50% tumor parenchyma disappeared due to necrotic and fibrotic foci.

IV degree – total tumor resorption.

- **Results of Clinical Investigations**

LLLT was performed in 41 patients with conformed diagnosis of IIa - IIIa st. breast cancer; control group included 40 patients. Distribution patients on age was from 37 till 76 years (Table 14).

According to the stage of disease patients were divided the following way (Table 15).

All patients had to undergo surgery operations. The great part of patients had Mammectomy by Holsted (LLLT group – 73,1%, in control group – 77,5%) (Table 16).

Parameters of "white" blood during 2 years after surgery show the following changes: in blood of patients who had LLLT leucocytes increased by 16,72% in comparison with initial level. In control group leucocytes decreased after surgery by 25,82% in comparison with initial level. After 3 months over surgery LLLT leucocytes normalized till normal level. In the control group leucocytes increased up to the initial level only after 2 years over surgery operation.

Increase of lymphocytes after LLLT added to surgery operation reached 39,16% over initial level of Lymphocytes. Increase of lymphocytes after surgery without LLLT reached them by 33,3% higher than initial level. Lymphocytes reacted on LLLT by increasing their count during 18 months after surgery.

The peculiarity of the reaction of monocytes on LLLT was their significant increase by 50% from the initial level during 6 months after 3 courses of LLLT. Laser therapy increased count of mono-

cytes up to 24 months after surgery. In the control group after surgery monocytes changes were non-significant, only in 12 months these data returned to the initial level.

Dynamics of the Immunoglobulins G fraction after surgery and LLLT could be characterized by increasing on 9,94% with the following decreasing of Ig G fraction to the normal level up to 6 months after surgery. In the control group after surgery decreased the number of Ig G lower than the normal data and only in 12 months these data returned to the normal level.

Reaction of the Immunoglobulins M fraction after surgery and LLLT increased during all observation period that lasted 12 months. In the control group their dynamics was difficult to explain because due to highly spread results in all patients.

Changes in Immunoglobulins A fraction after surgery and LLLT were non-significant. In the control group after surgery count of IgA decreased slightly.

Subpopulation of Trf lymphocytes after surgery and LLLT reacted by their increasing up to 89,65%. Subpopulation of Trf only after surgery was higher by 22,45% than the initial data. Using LLLT during 24 months increased these data by 37,14% in the average.

Subpopulation of Th increased after surgery and LLLT by 107,35%. Subpopulation of Th after surgery without LLLT increased their number by 29,4% higher than the initial level and only in 12 months returned to the initial level. In postoperative period LLLT increased count of Th up to 12 months after surgery, afterwards these changes were non-significant.

Reaction of LLLT on Subpopulation of Ts sowed more evident increase. After surgery with added LLLT their number increased by 82,5% higher from the initial level. Even in 2 years after surgery and LLLT the number of Ts kept by 90% from the initial level. In the control group after surgery without LLLT number of Ts decreased by 11,5% and after 3 months this data returned to the initial level. The assessment of the postoperative period and the number of early and late complications after LLLT and surgery are presented in (Table 17). Received analyses show that LLLT considerably decreases the number of postoperative complications in comparison to the controls.

More substantial histological investigations were done in 12 patients - 11 patients had invasion ductus cancer, 1 patient – colloid cancer.

In I group: 3 cases had no signs of treatment pathomorphosis, but in 2 cases of them were signs of limphoid-histiocytoid infiltration. In 1 patient limphoid-histiocytoid infiltration was more marked.

In II group: patients had predominantly the I-II degrees of curative damages: cancer nodes had necrotic foci, as a rule of a small size. In one case - in the necrotic zone microcirculatory disturbances (erythrostasis, hemorrage), cells detritus and large number of mitosis (>10 in the field of vision, magnification 400).

In III group: In 2 patients I-II degrees of curative damage was predominantly seen. In 1 patient with colloid cancer changes were cellule less "lakes" of mucin. However, the larger part of parenchyma was preserved, practically without significant dystrophic changes. In all 3 patients in cancer cells a large number of mitosis (>10 in the field of vision, magnification 400). In 1 case - changes in cancer cells were interpreted as the III degree of the damages: the larger part of the tumor parenchima was necrotized; in the preserved cells dystrophic changes were significant; cells were enlarged in their volume due to light vaculysation of cytoplasma; nuclei were picnomorphic (Figure 14).

The main criteria of efficiency treatment in all groups - is the prolongation of their lives. Analysis has shown that the survival in 10 years after the treatment was the following (Table 18).

As one can see after 5 years - LLLT increases the number of the survived patients with the IIa st. by 14,29% compared to the control group. With the IIIa stage - by 15,46%. The main criteria for the treatment - the number the patients without recurrences. After LLLT number of patients with IIa st. increased by 13,53% compared to the control group. In patients with IIIa stage - by 22, 35% comparing to the controls.

After10 years - LLLT increased the number of the survived patients with the IIa st. by 10,71% compared to the control group. With the IIIa stage - by 5,6%. The main criteria for the treatment - the number the patients without recurrences. After LLLT number of patients with IIa st. increased by 9,59% compared to the control group. Patients with IIIa stage - by 10,5% comparing to the controls.

Table 14: Classification of patients with IIa-IIIa st. breast cancer according to their age (number of patients).

Age	31-40	41-50	51-60	61-70	71-80
LLLT	4	16	18	3	_
Control	3	20	15	1	1

Table 15: Distribution of patients with IIa-IIIa st. breast cancer according to the stage of disease (number of patients).

Stage of disease	LLLT	Control group
II[a]	23	21
III[a]	18	19

Table 16: Classification of patients with IIa-IIIa st. breast cancer according to the type of surgery (number of patients)

Types of operations	LLLT	Control group
Mammaectomy by Holsted	30*	31
Mammaectomy by Patey	11	9
Sectoral resection of mamma with limphoidenectomy	1*	

*- one patient was done two surgery on both mamma glands.

Table 17: Postoperative complications of patients with IIa-IIIa st. breast cancer trated by LLLT (%).

Complications		LLLT	Control group
Suppuration postoperative wound		7,32% (3 patients)	12,5% (5 patients)
Limphorrea		4,88% (2 patients)	1 5% (6 patients)
Late postmammaectomy oedema	No	87,82%	77,5%
	Mild	9,75%	20%
	Severe	2,43%	2,5%

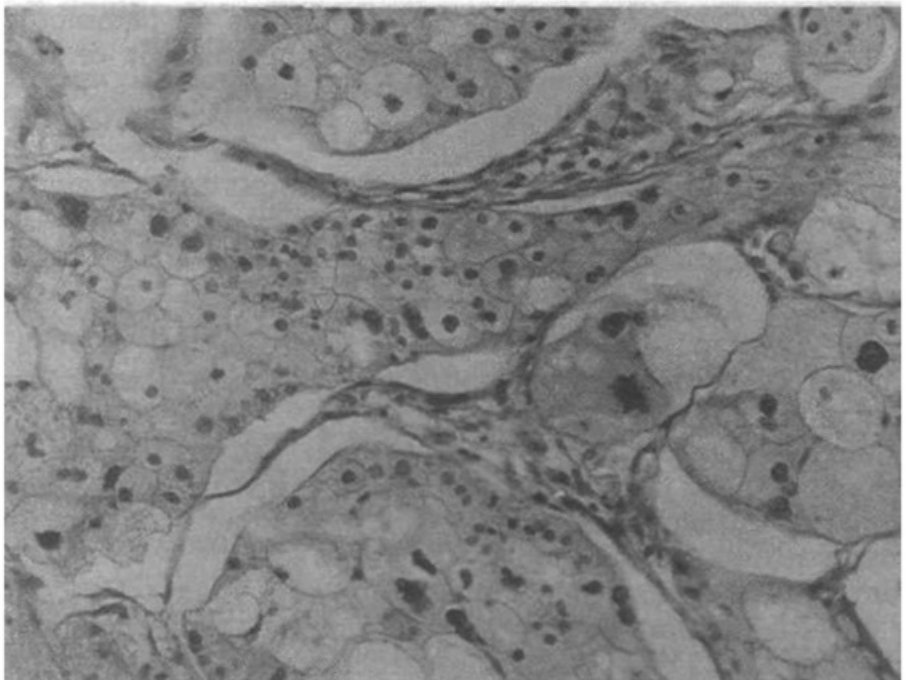

Figure 14: Invasive ductus breast cancer. Marked dystrophic changes in tumor cells. Cells is enlarged in their voilume due to the lights vacuolization of cytoplasma: nuclei are picnomorphic; pathologyc mytosys. Magnificaftion 400 (hematoxylin and eosin).

Table 18: Survival in patients with IIa-IIIa st. breast cancer treated by LLLT (%).

Stage	LLLT group				Control group			
	Number of survived		Without recurrences		Number of survived		Without recurrences	
	5 years	10 years	5 years	10 years	5 years	10 years	5 years	10 years
IIa								
	100%	86.90%	91,3%	82.60%	85,71%	76,19%	77,77%	66.6%.
IIIa								
	94,4%*	83,3%	82,35%	77,7%	78,94%	68,4%	60%	57,9%

* After 5 years - one patient died because of an accompanying disease but not from the cancer progressing.

- **Discussion of The Results**

The data obtained confirm our suggestions about the efficiency of laser therapy in oncologic patients. Our experimental study shows that manipulating with regimes and doses of laser beam one can get both the inhibition of the tumor growth and the stimulation of the immune system (2,3,10,20). The effect of LLLT has a multifunctional character. We consider that one can speak about the increase of the functional activity of cells of the immune system (3,7,9,10,) determined, by their quantitative growth (5), as well as about changes in cellular membrane transport and microcirculatory impairments (15).

Now, let us analyze how the results obtained correlate with the experimental and theoretic studies. During laser therapy one can observe a genera1 increase of leucocytes and lymphocytes number.

It can be explained how the release of these cells from the immune-producing organs and the organs in which these cells are

deposited. Where the stimulation of the functional activity of the immune cells begins. An interesting peculiarity of the immune response is that the lymphocytes react to laser irradiation almost one year after the surgery, while in the control group suppressive effect was seen up to 1,5-2 years (3, 10, 12). The increase of monocytes reaction in 6 months after the surgery signifies that along with immunity specific factors the macrophage system with its nonspecific character begins to act as well.

Changes in immunoglobulin fraction shows that laser beam mostly effects on IgG and Ig. M.

A great increase of IgG most likely means the increased release of antibodies. It can be caused both by the organism increased reaction to tumor growth and by changes in the antigenic structure. Besides, if IgG have receptors to T-killers, indirectly it means their increase. It is important to underline that the reaction of this class of Ig. to laser impact preserves 18 months after the surgery.

IgM react to LLLT immediately after the surgery and almost one year after it. If one can explain the increase of IgM immediately after the surgery as the reaction to surgical trauma, then the laser increase of IgM can be explained by the activation of B-lymphocytes system to which IgM have their receptors.

The peculiarity of T-lymphocytes reaction to laser irradiation lies on a reliable increase of T-rf number during the whole follow-up period (24 months). T-helpers were reliably increasing only one year after the surgery. Further sessions of LLLT did not cause reliable changes in this cell fraction. T-suppressors responded to laser therapy during the whole observation period; they increased by 54, 4% in average as compared to the initial data (10,12,13).

However, the most important, to our mind, is the fact that for this group of patients surgery was not such a powerful stress leading to the suppression of all protective system as it was for the patients from the control group. Thus, in the controls, the suppressive effect of the surgery was seen 12 months later: T-rf normalized only 12 month after the surgery; T-help. - in 12 months; T-suppressors - in 6 month (1,6).

Reduction of postoperative complications by 15,28% after LLLT is caused, to our mind, not only by the immunity stimulation, but by the local antiinflammatory effect of laser therapy as well. No doubt, various regimes of laser therapy depending on frequency and dose of irradiation produce various effects. Thus, adequate dosage can cause both the improvement of microcirculation at the irradiated site, if it is necessary, and its damage (16,18). Less late postmastectomy edema in the group of LLLT treated patients can be explained by the fact that lymphodrainage was compensated by better microcirculation and vessels formation after laser therapy. Thus, severe postmasectomy edema, which develops after mechanic injury of large lymphatic vessels, and collateral insufficiency are equal in the experimental group and in the controls because laser therapy in the given case could not be effective.

Histologic analysis confirmed the importance of LLLT application in treating immunocompetent zones, because in the intact tumor which was not irradiated with laser, one can see the increase of lymphoid-histiocytic infiltration in the tumor parenchima. Direct irradiation of tumor projection with the total dose 0,24-0,46 J/cm2 caused the I-II degree of curative pathomorphosis. Increase of the dose for the direct irradiation of tumour projection up to 0,46-1,53 J/cm2 confirmed the development of curative pathomorphosis of the I-II degree; in one case changes in a laser treated tumor were evaluated as curative pathomorphosis of the III degree (15,16,17).

The results of experimental and clinical studies have allowed us to explain the mechanism of action of laser therapy when exposed to various types of tumors and to form the basic principles of its use (3,4,5,6,7,8).

The long-term results of the use of laser therapy in breast cancer have confirmed our theoretical and practical developments. Our accumulated experience has allowed us to develop more effective treatment regimens. Over the past 10 years, we have significantly increased the pulse power to 100 watts and fundamentally changed the modes of using laser therapy in cancer patients (11).

5. Conclusion

1. Investigations of LLR effect on different types of implanted and spontaneous tumors have shown that this treatment can be used for tumor-static effect and for increasing life-span in animals.
2. Studies of the LLLR effect on inoculated tumors of various types have shown that LLLR can be used in combination with chemotherapeutic agents for achieving tumors-static action.
3. Changes in the accumulation of HpD and disturbances in its release when using laser therapy are caused by changes in permeability both in the capillary wall and in the cell membrane.
4. Laser therapy leads to stimulation of immunologic system in patients with IIa- Ia, III and IV st. breast cancer. That makes it possible to decrease a number of postoperative complications by 15,28 % relatively to control group and improves the quality of life.
5. Histologic study shows that direct irradiations of tumor projection may lead to appearance treatment pathomorphosis up to III st. degree (more than 50% tumor parenchyma disappeared due to necrotic and fibrotic foci).
6. Laser therapy improves 10 year results of patients survival with IIa st. of disease by 10,71% and with IIIa st. by 5,65% relatively to control data. After laser therapy use the cases without recidives increased by 9,59% in patients with IIa st. of disease and on 10,5% in patients with IIIa st. comparing to the controls.

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