Cu-LASER IRRADIATION MODULATION OF CYTOSTATIC ACTION OF 5-FLUOROURACIL AND ANTIOXIDANT ENZYMES ACTIVITY IN MICE BEARING MALIGNANT TUMORS

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In this paper we show a possibility of enchancing the antitumor and antimetastatic effect of 5-fluorouracil (5–FU) in C57BL/6 mice bearing Lewis lung carcinoma due to irradiation with the emission of Cu-*laser. The laser radiation increases superoxidismutase (SOD) activity in the blood plasma, the decrease of which is observed at tumor growth and cytostatic therapy. The SOD activity in a tumor decreases, on the contrary, at cytostatic and laser therapy as compared to that in untreated mice. The influence of laser irradiation on the antioxidant enzymes activity is likely to be one of the mechanisms responsible for the laser radiation ability to control the efficiency of cytostatic therapy.*

The basic research in the field of laser medicine introduced a low-intensity laser radiation (LILR) into the medical practice. The ability of lasers to stimulate photobiological processes leading to weakening of local inflammatory reactions, quicker wound healing, restoring of the disturbed adaptation mechanisms and hemodynamics is widely used.[8,12] A number of authors have shown the immunomodulating effect of irradiation from different lasers and its ability to influence the state of radical-free processes in organism.[2,6] At present the use of LILR in oncology is limited mainly by treating complications in various techniques of antitumor therapy.[4,5] In the literature there are ambiguous and sometimes even contraversial data concerning the LILR influence on the growth and metastatic spreading of tumors that can be explained by a variety of radiation spectra and modes of irradiation.[1,10] One of the promising approaches of antitumor therapy is considered to be the use of LILR in combination with classic methods of treatment, such as ray and cytostatic therapy. Recently the data concerning the ability of different laser radiation to enhance the efficiency of chemo- and radiotherapy have been obtained[14,17] experimentally.

At present there is a convincing evidence of the role of radical-free metabolites and lipid peroxidation in the mechanism of antitumor action of chemodrugs series and radiotherapy.[11,18] In this case, intensification of oxidation reactions causes the occurrence of side effects connected with toxic influence of reactive metabolites on the organs and tissues in an organism.[7,11] It is likely that correcting action of laser irradiation is connected with its ability to influence the activity of the main antioxidant enzymes responsible for the organism defence in conditions of an oxidation stress in cytostatic therapy.

No information appeared in literature about applying laser irradiation of yellow-green spectrum to oncologic patients. In our earlier investigations we have obtained a moderate antimetastatic and antitumor action of Cu-laser.[15]

The assessment of Cu-laser ability to enhance the efficiency of cytostatic therapy and influence the activity of antioxidant enzymes in the tumor and blood in mice bearing malignant tumor is given in this paper.

MATERIALS AND METHODS

Investigations were carried out in 160 C57BL/6 mice with transplanted syngenic hematogenic-metastasizing Lewis lung carcinome (LLC). The tumor was transplanted using a standard technique to the region of thigh subcutaneously in concentration of 1 mln of cells per mouse.

In the experiments we used a Cu-laser unit «Malakhit» generating pulses at a repetition frequency of 15 to 22 kHz. Two spectral lines, namely, green (510.6 nm) and yellow (578.2 nm) are in the laser emission spectrum. Irradiation at a dose of 30 J/m^2 to the tumor region (exposure of 1 min) was carried out daily during 5 days. A dose of 5-fluorouracil (5–FU) was injected intramuscularly 3 times at amounting to 25 mg/kg every other day. The cytostatic and laser therapy started on the 7th day after the tumor transplantation. The investigations were carried in 4 groups of mice: 1 – control-mice with a tumor without influence; 2 – mice with the tumor treated with 5–FU; 3 – mice with the tumor treated with laser radiation; 4 – mice with the tumor treated with 5–FU in combination with the laser irradiation. The tumor growth inhibition (TGI) was estimated by the following formula:

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$$TGI = (V_c - V_e)/V_c \cdot 100 ,$$

where V_c is the tumor volume in the control group, V_e is the tumor volume in the experiment. Dissemination of the metastatic process was determined by the metastatic spreading rate and the mean number of metastases to the lungs per mouse.

The sampling was conducted on the 18th to 20th day of the tumor growth after cervical dislocation of mice. For estimating the antimetastatic activity the index of metastases inhibition (IMI,%) was calculated by formula

$$IMI = 100 \%[(A_c \cdot B_c) - (A \cdot B)] / A_c \cdot B_c ,$$

where A_c and A are the number of mice with metastases in the control and experimental groups; B_c and B are the average number of metastases in the lungs in the control and experimental groups.

For a conclusion concerning the character of 5–FU interaction and laser irradiation in their combined application to be drawn the expected effect was calculated as follows:

$$A + (100 - A) \cdot B/100 ,$$

where A is the effect of the first action; B is the effect of the second action.[22] In case, the actual effect was equal to the expected one or even exceeded expectations, we drew a conclusion about the potentiating action of the agents.

The activity of antioxidant enzymes – glutathione peroxidase (GlP) and super-oxidismutase (SOD) in the tumor tissue and blood plasma was estimated in all mice. The activity of glutathione peroxidase was defined using spectrophotometry at the wavelength of 340 nm by a decrease in the NADPN concentration.

The principle of the method is based on NADPN oxidation in conjugated glutathion reductase reaction of reducing tertiary butyl hydroperoxide.[24] The principle of the method to determine the superoxidismutase consisted is that the superoxide anion-radical forming in the system of xanthine-xanthinoxidase reacts with nitroblue tetgasole resulting in forming the diphormazane absorbing laser radiation at the wavelength of 560 nm.[9]

The statistical data processing was performed using the non-parametric Wilkokson–Mann–Witney criterion.

RESULTS AND DISCUSSION

The experimental data given in Table I show that the treatment of tumor bearing mice with a cytostatic agent or laser irradiation as well as with their combination led to a considerable inhibition of the tumor transplant growth. It is seen that laser radiation obviously inhibits the tumor growth, however, to a lesser degree compared to 5–FU. The greatest inhibition of the tumor growth was observed in mice that received cytostatic and laser irradiation simultaneously (see Table I). Using the formula for estimating the effect of a combined chemo- and laser therapy we have shown that irradiation with a Cu-laser radiation enhances the therapeutic effect of 5–FU.

TABLE I. Inhibition of the Lewis lung carcinome in C57BL/6, mice treated with 5-FU in combination with Cu-*laser radiation.*

The tumor growth inhibition, %				
Days [x]		Laser	5–FU	Laser + 5–FU
10	1[xx]	12	44	45
	2	32	49	54
Average		22	47	50
12	1	41	53	71 **
	2	16	73	88.5 **
Average		29	63	80 **
14	1	37	51	60
	2	37	80	90.6 **
Average		37	65.6	75.5 **
16	1	40	60	69 **
	2	51.5	86.2	88.5
Average		46	73	79

[x] Days after the tumor transplantation;
[xx] 1 and 2 refer to two series of experiments;
*, ** The difference is reliable against the group treated with 5–FU (** $P < 0.05$; * $0.05 < P < 0.1$).

In all the mice groups there was noted 100% rate of metastases to the lungs. However, the average number of metastases per mouse in the group that received laser therapy or 5–FU taken separately was practically two times lower as compared to the control one. Use of 5–FU in combination with laser irradiation has led to a four-fold decrease in the number of lung metastases (Table II). The index of inhibition of metastatic spreading in mice treated with 5–FU only made 40% compared to 51% in mice subjected to laser irradiation the combined use of cytostatic and laser therapy decreased by 77% the level of metastatic spreading.

TABLE II. Mean number of metastases and the inhibition index in mice with LLC that were treated with 5-FU and Cu-*laser radiation.*

Group	Control	5–FU	Laser	Laser + + 5–FU
Number of metastases	8.6 + 1.5*	5.2 + 1.4	4.1 + 1.3	2.0 + 0.7*
Inhibition index	–	40	51	77*

* The difference is reliable as compared to 5–FU treated group.

Thus, the results obtained confirm the fact that the use of cytostatic in combination with Cu-laser irradiation leads to a more pronounced inhibition of the tumor growth and metastatic spread compared to cytostatic monotherapy.

It has been shown that the activity of superoxidismutase (SOD) in the tumor tissue in mice untreated was maximum and decreased when using 5–FU or LILR both separately and in a combination (see Fig. 1). As a result, there may occur weakening of the processes of superoxide anion-radical utilization in the tumor cells, and, as a consequence, an increase in their sensitivity to the radical-free disturbance.

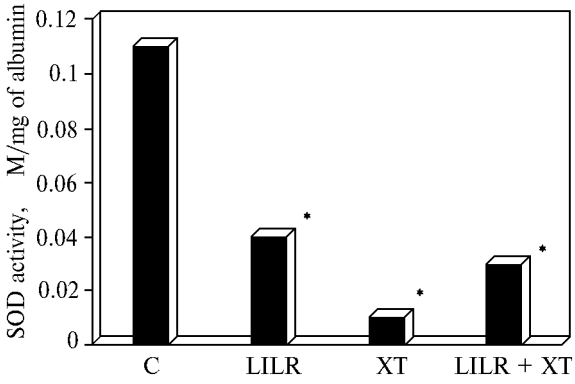

FIG. 1. SOD activity in the LLC tissue in C57BL/6 mice treated with 5-FU in combination with Cu-*laser radiation;* C – *mice with LLC that were not treated;* LILR – *those treated with* Cu-*laser radiation;* XT – *mice treated with 5–FU only;* LILR + XT – *mice treated with* LILR *and 5–FU.*
* *The difference is reliable compared to the group* C *($P < 0.05$).*

The highest SOD activity in the blood plasma is observed in intact mice. The growth of the tumor leads to a considerable decrease of the SOD level, and use of 5–FU inhibits its activity much stronger (Fig. 2) that points to the disturbance of the processes of antiradical defence in the organism of the tumor carriers and in cytostatic therapy. The combined influence of LILR and 5–FU increases the SOD activity practically to the level observed in the control group of intact mice (see Fig. 2). Similar values are observed in the group of 8 mice received LILR only that is indicative of its activating action on the state of the antioxidant defence indices. The GlP activity in the tumor tissue and blood plasma in the group under study did not change to a reliably detectable degree.

The data about LILR ability to enhance the efficiency of cytostatic and radiotherapy of experimental tumor were obtained by some researchers when applying helium-neon, infrared, rhodamine, arsenide-gallium lasers.[14,17] It has been shown that the modulating LILR effect is associated with its ability to cause a local hyperemia in the tumor tissue promoting the increase of the tumor cells sensitivity to disturbing influences.[18] When treating with 5–FU there is observed an increase in the blood viscosity, in this case the change of the blood circulation in the tumor may be accompanied by a decrease in cytostatic penetration into the tumor[21]; probably, the increase of the antitumor effect of 5–FU revealed in our investigations can be conditioned by hyperemia of the tumor tissue under the action of laser irradiation.

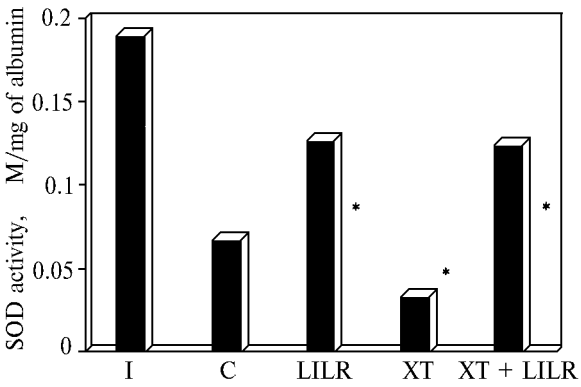

FIG. 2. SOD activity in blood plasma of C57BL/6 mice with LLC treated with 5–FU in combination with Cu-*laser radiation. Designations are the same as in Fig. 1;* I – *intact mice.* * *The difference is reliable compared to the group* I *($P < 0.05$) and* XT *($P < 0.05$).*

The antitumor and antimetastatic effect of LILR may be connected with modulation of the interrelations on the level the organism-tumor including its immunomodulating properties and influence on the activity of antioxidant systems. The experimental clinical investigations show that the development of a malignant tumor in the organism initiates the processes of lipid peroxidation.[3,13] In this case there is observed a decrease in the activity of enzymic and non-enzymic antioxidant mechanisms,[16] this process is aggravated by the radio- and chemotherapy conducted.[7,11,25] It has been shown that LILR favors increasing SOD activity in the blood plasma of the tumor carriers and considerably increases its level in mice treated with 5–FU. Similar rise of the SOD activity in the blood plasma and liver was observed by other investigators when applying helium-neon laser irradiation.[6]

One can find information in literature about increasing the antitumor activity of 5–FU when applying the antioxidant complex during the period of chemotherapy.[23]

It is known that one of the important multipurpose mechanisms of the tumor cells killing in radio- and chemotherapeutic effects and also with participation of the immunocompetent cells of the organism is considered to be initiation of the radical-free oxidation with a subsequent disturbance of the cellular membranes and DNA.[11,19,20] In this connection the LILR induced decrease of the SOD activity in the tumor, as one of the key antioxidant enzymes accelerating inactivation of

reaction products of the radical-free oxidation, may be considered as a factor reducing the tumor cells resistance to cytotoxic effects.

CONCLUSION

Thus, the results obtained show the ability of Cu-laser irradiation to increase the efficiency of 5–FU therapeutic effect. Laser irradiation increases the SOD activity in the blood plasma considerably decreased with the tumor growth and cytostatic therapy and, on the contrary, decreases the SOD activity in the tumor tissue. The influence of laser irradiation on the activity of antioxidant enzymes can be one of the mechanisms responsible for its ability to increase the efficiency of cytostatic therapy.

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