HEALING OF EXPERIMENTAL WOUNDS IN INTACT ANIMALS AND IN ANIMALS BEARING TUMORS UNDER THE ACTION OF A COPPER-VAPOR LASER RADIATION

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We present some results of studying the process of healing experimental wounds in 130 rats under the action of copper-vapor laser radiation of both green and yellow lines. The characteristics studied were the speed of healing and morphological changes of wounds in intact animals and in animals with the interwoven Pliss lymphosarcoma. As a result of this study, we have established that emission of a copper-vapor laser stimulates the regeneration process and inhibits the growth and metastasizing of tumors.

The necessity of studying the effects that may occur under the action of copper-vapor radiation is partially caused by the lack of literature data on how that kind of irradiation may affect the regeneration. However, one may find in the literature some data on the influence that radiation of a He–Ne laser, a semiconductor laser, and of a laser emitting in the UV region may exhibit on the clinics of processes in wounds. The data of such studies have confirmed the stimulating role of these emissions in the regeneration process due to improved, under the irradiation, blood circulation in the tissues around an experimental wound and due to intensified cell corpulence reaction. Along with the increased functional activity of the connective tissue cells there occurs enhancement of the epithelium cells' proliferation that favors the wound healing.[2,3] In the majority of experimental animals there is observed regeneration of skin as of organ with the hair bulbs and glands. The laser radiation modifies the cellular composition of the wound excretion by lowering the number of granulocytes while increasing by 10 to 12% the amount of macrophagocytes and histiocytes.[1,4]

At the same time only few papers may be found in literature concerning the effect of metal-vapor and, in particular, of copper-vapor laser on the regeneration of tissues. Thus I.M. Baibakov mentioned in his paper,[1] where he compared the effects of He–Ne laser, copper-vapor laser, gallium arsenide laser and a laser emitting in the UV region, that the copper-vapor laser activates the cells of connective tissues more efficiently than other lasers. The changes in ultrastructure of fibroblasts are indicative of the enhancement of their specific function. These changes are observed as extended profiles of the cellular structure of Golgi complex endoplasm.

In this connection, we think that it to be timely studying the influence of the copper-vapor laser radiation on the regeneration processes in wounds in intact animals and in animals bearing tumors, as well as looking for most efficient irradiation modes.

MATERIAL AND METHODS

The study of the wound regeneration on the background of irradiation with a copper-vapor laser has been done in 50 noninbred male rats. We removed from rats, under the hexenal anesthesia, a 2 by 2 cm piece of dermis to fascia in the upper third portion of a thigh. On the next day after this surgery we started irradiation of the wound with the low-intensity emission from a pulsed copper-vapor laser MILAN–1. The irradiation lasted ten days. This laser simultaneously delivers pulses at two wavelengths, yellow at 578.2 nm and green at 510.6 nm, with the intensity ratio 1:3. In these experiments we tried four regimes of wound irradiation. In the first one the wound has been being irradiated with the green emission at the power level of 50 mW and total dose of 0.5 J/cm^2, in the second one with the yellow emission of the same power and dose. In the third and fourth experiments we used those emissions with doubled power and dose levels, that is, at 100 mW and 1 J/cm^2, respectively. The reference group comprised also wounded animals that were not irradiated. The area of wounds has been measured every day until the complete skinning over with epithelium. The daily decrease in the wound area has been calculated by the following formula:

$$\Delta S = 100\ (S - S_n)/S\ t\ ,$$

where S is the area of wound as measured earlier, S_n is the area of the wound at a current measurement, and t is the number of days between the measurements.

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To measure the wound area we first cover it with a piece of sterile cellophane and then mark the wound circumference with a pencil. Thus obtained picture of the wound is then transferred onto the millimeter graph paper and its area counted in square millimeters.

To study the effect that may occur in the wound skinning over in tumor bearing animals at the irradiation by a low-intensity laser radiation, as well as the tumor behavior under the irradiation we have arranged an experiment with a group of 80 males of white noninbred rats. These animals have been inoculated, following a conventional technique, with the interwoven Pliss lymphosarcoma culture. After the tumor nodes grew to be accessible the animals were wounded according to the above technique. The control group comprised wounded animals with the interwoven tumor that did not receive lasertherapy. The experiment has been carried out with two groups of animals, one of which received the irradiation dose of 1 J/cm^2 while the other one 0.5 J/cm^2. The tumor growth dynamics was estimated using three mutually orthogonal dimensions of the tumor, A, B, and C. The tumor volume is calculated by the following formula: $V = A \cdot B \cdot C \cdot \eta / 6$. The degree of metastasizing has been being assessed by the mean number and mass of metastases per one animal.

EXPERIMENTAL RESULTS

It was established in these experiments that laser radiation of 510.6 nm wavelength and 50 mW mean power exhibits an essential stimulating effect, at a dose of 0.5 J/cm^2, on the wound skinning over in intact animals. For instance, the daily percentage of the wound area decrease is several times higher as compared to that in animals from a control group that were not irradiated. Thus, on the 2nd day $\Delta S_{\text{contr}} = 14\%$, $\Delta S_{\text{exper}} = 21\%$. The corresponding figures for the 3rd day were 8 and 17%, 9 and 30% on the 5th day, 10 and 17% on the 9th day, and on the 15th day they were 26 and 40%, respectively. In the group of animals that were irradiated with the yellow emission of 578.2 nm wavelength these figures were a little bit lower. Thus, ΔS_{exper} values measured on the 5th, 9th, and 15th day were 8.4, 13, and 35%, respectively. An increased, up to 1 J/cm^2, dose of irradiation produces a more pronounced effect, especially in the second half of the wound skinning over (see Table I).

TABLE I. Skinning over of wounds in animals under the action of low-intensity laser radiation.

The number of experimental group	The parameters studied		The percentage of the wound area decrease				
			Days of observation				
	P, mW	W, J/cm^2	2nd	3rd	5th	9th	15th
1.0	–		14.0	8.0	9.0	9.6	2.6
	Green line						
1.1	50	0.5	31.0	22.0	30.0	17.0	4.0
	Yellow line						
1.2	50	0.5	15.0	17.0	21.0	12.0	3.5
	Green line						
1.3	100	1	36.0	24.0	37.0	18.0	14.0
	Yellow line						
1.4	100	1	21.0	17.0	22.0	13.0	3.5

Time and character of the skinning over process in the animals with the interwoven tumor were the same as in the intact animals. At the same time we have noticed an antitumor effect of the copper-vapor laser radiation.

Thus in the group of animals that received a 1 J/cm^2 dose of the green radiation there was achieved a 59% inhibition of the tumor growth with simultaneously inhibited metastasizing. The metastasizing frequency dropped down to 63% of that in the control group. The mean number of metastases per one animal was 1.0 against 2.3 in the control animals. The mean mass of metastases has also essentially decreased (see Table II).

TABLE II. Influence of copper-vapor laser radiation on the growth of Pliss lymphosarcoma when irradiating the experimental wounds in animals.

Experiment version	Inhibition of the tumor growth, %	Metastasizing frequency, %	Mean number of metastases	Mean mass of metastases, mg
Control	–	100	2.3	733 ± 15
λ – 510.6 nm; W – 1 J/cm^2	59	63	1	213 ± 25
λ – 578.2 nm; W – 1 J/cm^2	–	33	0.38	102 ± 9

In the second experimental group that were irradiated with yellow light of the same power and dose no inhibition of the tumor growth was noticed while a more pronounced inhibition of the metastasizing occurred. The metastasizing frequency dropped down to 33% of that in the control group, with the mean number of metastases being 0.38 per animal and the mean mass of 102 ± 9 mg against 733 ± 15 mg in the control animals. To reveal morphological changes that may occur in the skin wound under the laser irradiation we have microscopically studied the bioptic samples.

In seven days we could identify granulation, though of different ripeness, in the experimental and control animals. In the control animals we could see many blood capillaries of various size and shapes that were overfilled with blood and separated by a thick cellular infiltrate. The latter consisted of lymphocytes of different ripeness, plasmocytes, eosinophilic leukocytes, histiocytes, and singular fibroblasts.

The above cellular elements were concentrated in the swell fluid. No fibrous structures were identified in the intercellular space. In rats that received laser irradiation the granulation was more ripe and this resulted in a changed cellular composition and intercellular substance. In this case there was observed a lower number of lymphocytes, plasmatic cells, and histiocytes, while increased amount of fibroblasts. Certain fraction of fibroblasts formed tape-shaped fascicles and thin collagenous fibers. On the 14th day only inessential growth of fibroblasts has been observed in control animals (compared to a previously recorded) with no noticeable formation of fibers. On the contrary, in the experimental animals by this time there occurred the formation of connective tissues with a typically fine-fibered structure, accompanied by the presence of ripe fibroblasts and fibrocytes. It is also characteristic of the process in the experimental animals that only moderate swell and insignificant amount of lymphoid infiltrate around the blood vessels are observed. In this case the surface of the connective tissue is covered with islands of a typically multilayer epithelium.

Thus, it is seen that skinning over of the dermic wound in animals that underwent laser irradiation occurs due to the growth of granulation into a fibrous tissue, as it is in the control animals. However, in the former case the growth of granulation into a fibrous tissue occurs much faster. No reliably identified differences in the wound skinning over have been noticed in animals from the experimental groups.

CONCLUSIONS

The experiments carried out have shown that the low-intensity laser radiation of the wavelengths at 510.6 and 578.2 nm and the doses of 0.5 and 1 J/cm^2 makes the regeneration processes in wound in intact and tumor bearing animals more active and favors inhibition of the tumor growth and metastasizing. For these reasons it is worth considering to use such an approach to treatment of wounds and inflammation in oncological patients.

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