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Cellular Alterations Upon IR-Laser (890 nm) Exposures, *In Vivo*

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Exposure of cultured cells and small animals to ionizing radiation as well as irradiation of cultured cells with He-Ne laser can cause changes in the functional condition of plasma membranes. The ionizing radiation-induced cell membrane alterations have been determined after either partial or local exposures. The aim of the present study was to reveal whether the local laser treatments cause a general, distant, so called „abscopal" effect measured at cellular level, when the laser treatment is intended as a stimulatory procedure. The biological effect of infrared laser (mean power of 5 Watts, 150 Hz frequency, 890 nm wavelength) was demonstrated through ^{3}H-concanavalin A binding by blood cells of daily irradiated (altogether 10 exposures) oncological and non-oncological patients as well as by changes in the proliferation of bone marrow cells of whole body gamma-irradiated (4 Gy) rats, partially laser-treated. The lectin binding of lymphocytes of oncological, as well as ischaemic heart disease patients was increased immediately after the first laser treatment. However, it was decreased after completion of the full course. In cases of inflammatory diseases the test parameters were either unchanged or decreased as compared to their self-control values. The platelets and erythrocytes did not react in any group. Gamma irradiation caused a deep inhibition of proliferation of rat bone marrow cells. The number of fibroblast colony-forming units (CFU-F) could be increased again if the animals were partially exposed to laser. Laser irradiation of one of the femurs led to some recovery of CFU-F values in the exposed as well as unexposed femur. Thus, local infrared laser treatment induces abscopal effects on the cell membrane and cell proliferation characteristics. (Pathology Oncology Research Vol 4, No 1, 22–26, 1998)

Key words: IR-laser, gamma irradiation, abscopal cellular effects, cell membranes, cell proliferation

Introduction

The wide use of lasers in experimental and clinical medicine requires detailed information on the mechanisms of their biological effects. To understand laser-induced tissue alterations during application in surgery as well as in other clinical treatments, e. g. tissue stimulation, approach by cell biological studies is promising.

The aim of the present investigation was to study the cell membrane alterations of blood cells obtained from both oncological and non-oncological patients submitted to local low power infrared (IR) laser treatment, with the intention of beneficial stimulation of tissue reactions. According to our experience a lectin-binding technique is a sensitive method for revealing cell surface changes,[11] therefore this was used in the experiments.

Additional experimental work was also carried out to study the proliferative capacity of rat bone marrow cells upon local laser treatment of the previously gamma-irradiated animals.

Materials and Methods

Patients and preparation of cell fractions from blood samples

Citrated samples of blood were collected from laser-exposed patients with various locations of tumors of different cell composition; patients suffering from inflamma-

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Table 1. **Analysis of variance for the lectin-binding activity of blood cells originated from 4 oncological patients at the beginning and end of therapy with IR-laser of low power (as % of self-control, i. e. a lectin-binding activity was measured for the cells of patients before each laser treatment).**

Blood cells and period of sampling	*Time after laser treatment (hrs)*	*Lectin-binding activity (mean ± SD, %)*	*F**	*Significance α = 5%*
Platelets				
at the beginning	1	110 ± 20	8,94	9,28
after 10 days	1	70 ± 12	18,00	10,13
at the beginning	4	98 ± 15	2,23	9,28
after 10 days	4	95 ± 16	0,04	10,13
at the beginning	24	83 ± 11	0,75	9,28
after 10 days	24	139 ± 22	5,34	10,13
Lymphocytes				
at the beginning	1	145 ± 19	0,72	9,28
after 10 days	1	101 ± 9	10,45	10,13
at the beginning	4	150 ± 21	0,64	9,28
after 10 days	4	112 ± 3	18,51	10,13
at the beginning	24	196 ± 34	0,93	9,28
after 10 days	24	116 ± 11	36,59	10,13
Erythrocytes				
at the beginning	1	106 ± 10	1,30	9,28
after 10 days	1	102 ± 8	0,10	10,13
at the beginning	4	95 ± 19	0,22	9,28
after 10 days	4	111 ± 8	0,21	10,13
at the beginning	24	106 ± 20	6,67	9,28
after 10 days	24	101 ± 17	0,07	10,13

*The significant differences at 5% level are underlined, n = 4.

tory diseases, i. e. pneumonitis and pyelonephritis, as well as patients with ischaemic heart disease.

One-step separation of various blood cells (platelets, lymphocytes, erythrocytes) was performed on a Ficoll-Uromiro discontinuous gradient containing three layers (specific gravities: 1.048; 1.060; and 1.077 g per ml) as described earlier.[11] The blood samples were obtained before the laser treatment (control) then 1, 4 and 24 hours after the first as well as the tenth (last) exposures.

Lectin-binding procedure

The binding of lectin, tritiated concanavalin A (^{3}H-ConA, Amersham) to the surfaces of platelets, lymphocytes and erythrocytes was carried out in vitro according to our method published earlier.[11] The binding with 37 kBq per ml labelled lectin lasted for 10 minutes at room temperature. The unbound ^{3}H-ConA was removed by repeated washings then the level of bound radioactivity was measured in a liquid-scintillation spectrometer. For each laser treatment, the results related to laser effect were compared to the control values obtained just before laser treatments (self-control) and expressed in per cents.

The analysis of variance was used to evaluate the results obtained at the beginning and end of the laser-treatment course as well as at various time-points after laser treatment. Taking into consideration the significance of possible differences in means both among patients and the periods of laser therapy, two-way analysis of variance was applied. Thus, the sources of variability were patients, laser treatments and random variations.

Bone marrow cultures

Wistar male rats of 160–180 g weight were used for testing CFU-F formations. The femoral bone marrow cells were cultured in medium consisting of Parker 199 solution, 20 per cent foetal calf serum and antibiotics. The cell cultures were incubated at 37°C in the presence of 5 per cent CO_2 for 10–12 days.[2,3]

After fixation of the monolayers, colonies of 50 cells were counted. Finally, CFU-F values were determined in the control (untreated) rats, in the animals following whole-body gamma irradiation (4 Gy) only, as well as in rats receiving whole-body gamma-irradiation, then submitted 4 times to IR-laser treatment on one of the femurs (right), one exposure per day.

Laser- and gamma irradiation

All oncological patients were exposed to laser before the specific treatment (chemo- and radiotherapy). The non-oncological patients were exposed to laser in the course of therapy. The treatment with IR-laser of low power was licensed by the Russian Ministry of Health and performed with the consent of the patients.

The IR-laser exposure of the patients was performed by „UZOR" apparatus (Kaluga, Russia) with mean power of 5 watts, frequency of 150 Hz and wave-length of 890 nm. This Ga-As IR-laser works with impulse regime: one impulse lasting 50 ns; the divergence of bundle angle being 15°; the depth of laser penetration in soft tissues approximately 8 cm. The distance between the skin surface and the laser source was 1 cm, the duration of each treatment was 20 minutes. The reflexogenic zones of the respective organs of patients were exposed 10 times (one exposure per day).

The whole-body gamma irradiation of rats was performed by „LUCH" apparatus using doses of 4 Gy with dose rate of 0.5 Gy per min. After the exposure, the right femurs of animals were additionally treated with IR-laser: 4 times, one exposure per day. The duration of each treatment was 6 min 24 sec.

***Table 2.* Analysis of variance for the lectin-binding activity of blood cells originated from non-oncological patients (ischaemic heart disease, pneumonitis, pyelonephritis) at the beginning and end of therapy with IR-laser of low power (as % of self-control, i. e. a lectin-binding activity was measured for the cells of patients before each laser treatment).**

Disease, blood cells and period of sampling	*Time after laser treatment (hrs)*	*Lectin-binding activity (mean SD,%)*	*F**	*Significance $\alpha = 5\%$*
Ischaemic heart disease (n = 10)				
Platelets				
at the beginning	24	85 ± 27	0,76	5,12
after 10 days	24	120 ± 23	2,12	3,18
Lymphocytes				
at the beginning	24	130 ± 30	2,48	5,12
after 10 days	24	116 ± 19	3,53	3,18
Erythocytes				
at the beginning	24	104 ± 11	0,99	5,12
after 10 days	24	148 ± 23	2,45	3,18
Pneumonitis (n = 5)				
Platelets				
at the beginning	24	88 ± 6	1,67	7,71
after 10 days	24	86 ± 22	0,14	6,39
Lymphocytes				
at the beginning	24	99 ± 11	0,63	7,71
after 10 days	24	91 ± 5	0,15	6,39
Pyelonephritis (n = 4)				
Platelets				
at the beginning	24	71 ± 33	8,79	10,13
after 10 days	24	82 ± 28	0,22	9,28
Lymphocytes				
at the beginning	24	59 ± 25	0,79	10,13
after 10 days	24	68 ± 19	0,10	9,28

*The significant difference at 5% level is underlined

Results

Effects of low-power IR-laser on the lectin-binding ability of blood cells of oncological patients

The lectin-binding abilities of separated platelets, lymphocytes and erythrocytes were determined before each laser treatment as self-control levels. Laser-induced changes were examined 1, 4 and 24 hours after the 1st and 10th (last) exposures. The alterations expressed in per cent of controls are summarized in *Table 1*. Although the individual values were scattered in a rather large extent as expected, the difference among the reactivities of cell types tested was unambiguous. After the first laser treatment, significantly increased binding capability of lymphocytes could be observed for all time-points, while the membranes of platelets and erythrocytes remained practically unchanged. After 10-day laser therapy, the reactivity of lymphocytes returned to the control values, the response of erythrocytes stayed on the same level as at the beginning.

The differences between the post-irradiation values for 1, 4 and 24 hours were found to be significant in several cases at 5% level (see the underlined values in *Table 1*). Changes occurred in the platelet population only: the amount of bound lectin decreased in the first hour then gradually increased and - after 24 hours - overrode the control level (the difference was significant only for 1-hour post-irradiation period).

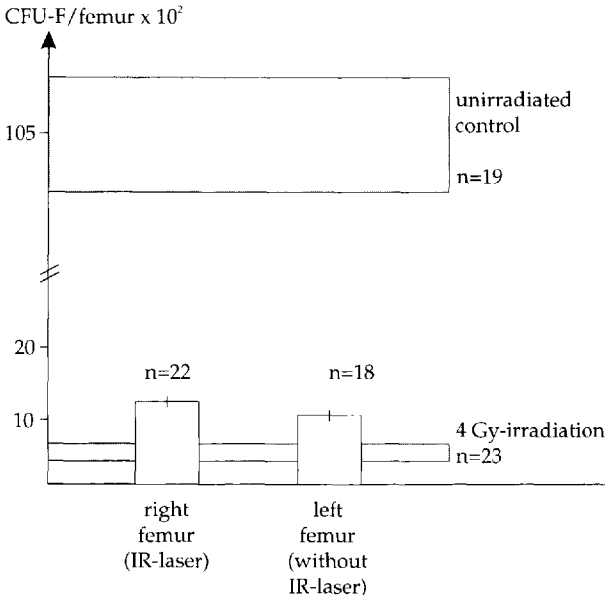

Figure 1. *Numbers of CFU-F$_s$ in femoral bone marrow of rats. The following animal groups were examined: untreated (control), 4 Gy-irradiated, 4 Gy-irradiated and right femur laser-treated. The laser treatments were performed 4 times (one treatment per day). For culturing, the suspensions of bone marrow cells were prepared on the 4th day after gamma irradiation. Mean values ± SD.*

Effect of low-power IR-laser on the lectin-binding capacity of blood cells of non-oncological patients

The blood cells of patients with ischaemic heart disease, pneumonitis and pyelonephritis were examined beginning and at the end of laser therapy, 24 hours following the treatments. The results are given in *Table 2*. In the cases of patients with ischaemic heart disease, the ConA binding of lymphocytes was only increased at the beginning of the treatments. At the end of therapy, the reactivity of other cell types was also enhanced. In the cases of inflammatory diseases the values did not differ significantly from the control levels or, even decreased amounts of bound radioactivity were measured.

Changes of rat CFU-F values upon laser exposures

The whole-body gamma irradiation of rats with a dose of 4 Gy depressed the proliferation of bone marrow cells obtained from femurs *(Figure 1)*. If one of the femurs (right) of such rats was additionally exposed to laser (4 times, one exposure per day), a well detectable slight stimulation was observed both in the laser-treated and counterlateral untreated femur.

Discussion

The favourable stimulatory effects of various laser types on the healing process of different superficial injuries (e. g. ulcus cruris, burns, erosions) are well known.[7,8] It is also known that deeply penetrating IR-laser has direct effect on tissues, cells and their membranes. There have been several reports regarding cell membrane alterations upon laser treatments.[1,6,9,14] The present work has demonstrated changes on the cell surfaces of blood cells following application of IR-laser, in vivo. The lectin-binding technique was considered as a sensitive procedure for the detection of ionizing radiation-induced alterations on platelets, lymphocytes and erythrocytes observed in vitro and in vivo.[10,11] Earlier we found increased amounts of surface negative charges on laser-exposed human fibroblasts.[9] Now, the increased ConA binding of lymphocytes of laser-exposed oncological patients was detected.

In the non-oncological cases, the lymphocytes of patients with ischaemic heart disease also had increased levels of bound ConA. In this group the elevated values were measured even for the erythrocytes, at the end of laser therapy. The cells from patients with inflammatory diseases could be characterized mainly by the decreased values of bound radioactivity.

The most probable explanation for the discrepancies among diseases of various nature is the different endogenous environmental conditions of the cells within the blood. Obviously, the changes on blood cell surfaces can not only be related to the direct effects of laser, but may probably also be attributed to the effects of biological mediators released from the irradiated cells and tissues.

The abscopal stimulation of bone-marrow cell proliferation assumed this explanation. Similar observations have already been published for UV irradiation.[12,13] It has been found that IR-laser exposure induces the release of IL-1 from rat peritoneal macrophages.[5] According to other authors, the treatment of cultured human peripheral blood mononuclear cells with He-Ne laser caused the increased release of various cytokines (after 30 min of postirradiation), followed by a significant decrease.[4] Obviously, the quality of mediators, their quantities and proportions would determine the final effects. Presumably, the laser-induced cytokines in case of oncopathology, ischaemic heart disease differs from that of inflammatory diseases like pneumonitis or pyelonephritis leading to various alterations on cell surfaces of platelets, lymphocytes and erythrocytes, which could be detected by lectin-binding.

Conclusions

The biological effects of local infrared laser irradiation was shown by lectin-binding of blood cells of irradiated oncological and non-oncological patients as well as by changes in proliferation of rat bone marrow cells previously suppressed with 4 Gy of whole-body gamma irradiation. The lectin binding of lymphocytes of oncological or ischaemic heart disease patients increased immediately after the first laser treatment. It decreased or was normalized, however, during the full course of laser treatment. In patients with inflammatory diseases no alterations of lymphocyte membranes could be observed as compared to the self-control values. The membranes of platelets and erythrocytes particularly did not react to the local laser treatment.

The number of CFU-F could be increased by laser in the rat bone marrow cells of femurs after previous suppression by 4 Gy whole-body gamma irradiation. The elevation of CFU-F values was detected even in the femur unexposed to laser.

It was concluded that local IR-laser irradiation induces abscopal effects as detected on blood cells and in bone marrow originated CFU-F parameter. It is suggested that the alterations might be attributed to the release of biological mediators like cytokines.

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References

1. *Burt JM, Strahs KR, Berns MW:* Correlation of cell surface alterations with contractile response in laser microbeam irradiated myocardial cells. Exp Cell Res 118:341-351, 1979.
2. *Friedenstein AJ, Latzinik NV, Gorskaya UF et al:* Radiosensitivity and postirradiation changes of bone marrow clonogenic stromal mechanocytes. Int J Radiat Biol 39:537-546, 1981.
3. *Kolesnikova AJ, Konoplyannikov AG, Hendry YH:* Differential sensitivity of two predominant stromal progenitor cell subpopulations in bone marrow to single and fractionated radiation doses. Radiat Res 144:342-345, 1995.
4. *Konoplyannikov AG, Konoplyannikova OA, Kaplan VP et al:* Radiomodified effect of IR-laser irradiation. In „Problems of modern oncology. 4 All-Russian Oncologica Congress, Rostov-on-Don. Book of abstracts, pp. 199-200, 1995.
5. *Jeliseenko VJ, Ryazhsky GG, Vorobyov SV et al:* To the question on low power laser photoacceptors. In „News in laser medicine". Int. Conf., Brest, 13-15 November 1991. Book of abstracts. Moscow, p. 93, 1991.
6. *Kitzes M, Twiggs G, Berns MW:* Alteration of membrane electrical activity in rat myocardial cells following selective laser microbeam irradiation. J Cell Physiol 93:99-104, 1977.
7. *Kovács L:* The stimulatory effect of laser on the physiological healing process of portio. Lasers Surg. Med. 1:241-252, 1981.
8. *Kovács L, Tisza S:* Treatment of ectopium with low put He-Ne laser. Neoplasma 28:351-354, 1981.
9. *Kubasova T, Kovács L, Somosy Z et al:* Biological effect of He-Ne laser: Investigations on functional and micromorphological alterations of cell membranes, in vitro. Lasers Surg Med 4:381-388, 1984.
10. *Kubasova T, Köteles GJ, Simbirtzeva LP et al:* Use of lectin-binding technique as a tool to assess the radiation dose. Radiobiol Radiother 27:783-91, 1986.
11. *Kubasova T, Köteles GJ, Varga L:* Surface alterations of mammalian cells upon ionizing radiation as detected by lectin-binding technique. II. Binding of concanavalin A by human blood cells X-irradiated in vitro. Int J Radiat Biol 40:187-94, 1981.
12. *Samoilova KA, Mironova AP, Artsishevskaya RA:* Release of substances from the human blood lymphocytes after UV irradiation (254 nm). Tsitologiya 26:102-108, 1984.
13. *Samoilova KA, Zaritsky AYu, Stankevich ER et al:* Effect of ultraviolet-irradiated donor blood on the human bone marrow colony formation in vitro. Tsitologiya 27:488-492, 1985.
14. *Uzdensky AB:* On selectivity and locality of the effects upon the laser microirradiation of cells. Citologija 10:1119-1132, 1982