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ORIGINAL ARTICLE

Morphological changes in human skin melanoma treated by high-energy pulsed neodymium laser radiation

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Summary

Purpose: *The neodymium (Nd) laser irradiation has been successfully applied to the treatment of slightly elevated skin melanoma. At the same time the histologic aspects of such a treatment have not been precisely investigated. The aim of this study was to retrace the histological features in human primary cutaneous melanomas after 1060 nm high-energy mono pulsed Nd laser treatment in the dynamic healing of the affected tissues.*

Methods: *Histologic analysis of cutaneous melanomas irradiated by Nd laser was carried out. Tissue specimens were taken before and immediately after exposure to laser and 1 hour, and 1, 2 and 3 days afterwards. Also the wounds that appeared after the scab fell off and the scars formed following laser irradiation were also the subject of histologic analysis at 2, 4 and 6 months post-treatment.*

Results: *The Nd laser irradiation caused coagulation necrosis of melanoma, epidermis and dermis with skin appendices and superficial layers of subcutaneous fatty tissue. Foci of laser destruction were characterized by strict locality and efficient separation from the adjacent tissues, by the presence of stasis, thrombosis and coagulation of blood and lymphatic vessels. There was an increase of lymphocytes, macrophages and histiocytes in the area damaged by laser as well as in wounds and scars.*

Conclusion: *The pulsed Nd laser induces acute photothermal damages of melanoma tissue, which differs from the usual thermal lesions and the most critical difference of the effect of this modality is gain of immunocompetent cells in the affected tissue after laser beam application.*

Key words: cutaneous melanoma, laser radiation, morphological changes, neodymium laser, treatment

Introduction

The high-energy pulsed Nd laser irradiation has been advocated as an alternative therapeutic modality for flat and slightly elevated cutaneous melanoma [1-3]. There is a number of references concerning morphological changes in normal tissues, experimental tumors, skin cancer and cutaneous metastases of malignant melanoma irradiated by different types of lasers [4-9]. At the same time there is no information regarding pathological changes in the skin of primary melanoma subjected to pulsed Nd laser irradiation. Owing to this lack of information we planned to examine the character of pathological changes in human primary cutaneous melanoma treated by high-energy pulsed radiation of Nd laser as well as in the wounds that appeared after the scab fell off and the scars formed in the place of irradiated tumors.

Methods

Eighteen patients with biopsy-confirmed primary cutaneous melanoma were included into this study. Melanomas were located on the skin of trunk and upper and lower limbs and clinically were tumors elevated over the skin surface by 1-3 mm. Histologically, they were epithelioid cell (n=9), spindle cell (n=2) and mixed cell (n=7) melanomas.

The histological evaluation of cutaneous melanomas was performed before irradiation and immediately after the tumor exposure to 1-2 pulses of laser radiation

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and also after 1 hour, as well as 1, 2 and 3 days after laser treatment according to our methodology [3]. The source of radiation was two solid state high-energy Nd laser installations (Pulsar - 1000 and its modification Pulsar - 1000M, Russia) working in mono pulsed mode. The wavelength was 1060 nm, the pulse duration was 1 and 4.5 ms, the maximum pulse energies were 700 and 1000 J, and the energy density in the pulse ranged from 350 to 450 J/cm^2, the intervals between pulses were 50-90 sec, and the light spot diameters were 0.5-1.5 cm. Tumor irradiation by laser was carried out under local anesthesia with 2% solution of lidocaine hydrochloride. Also in 5 patients, the smears of the wounds that appeared after the scab fell off were subjected to cytological study. In addition, in 10 patients histological studies were carried out of the scars formed following laser irradiation in the place of irradiated melanomas at 2, 4 and 6 months post-treatment. The incision biopsy of tumors was carried out before laser irradiation by forming a circular blocking barrier by means of a laser beam [3,10,11] that prevented possible dissemination of tumor cells via blood and lymph vessels. Each period of examination was accompanied by 3-5 specimens. The histological samples were fixed in 10% formalin and serial sections with a slice thickness of 5 microns were embedded in paraffin. The histological sections and cytological smears were stained with haematoxylin and eosin (H&E) and then were analyzed via light microscopy.

Results

Histological study of cutaneous melanoma specimens immediately after laser irradiation revealed tissue necrosis with clear margins. The tumors had completely lost their original structure and appeared to be a detritus of coagulated cells with numerous fissures and lacunas of different sizes. Next to the necrosis area dystrophic changes both in the nuclei and in the cytoplasm of the cells with different degrees of intensity, as well as fibrous tissue destruction were detected (Figure lA). The destruction of the cells' nuclei was characterized as pycnosis for they were shrunk and hyperchromatic. There were also single and numerous confluent vacuoles in affected nuclei as well although such nuclei did not become hyperchromatic and stayed colorless, their form usually being irregular.

The most typical change of the cytoplasm was its complete disappearance and it was particularly so at the area adjusted to laser beam hit. The entire cell revealed mostly an elongated spindle shape. There were numerous single and huge confluent vacuoles in the destructed cytoplasm so that in some cells the whole cytoplasm had become desolate and the nuclei looked like suspended in the emptiness surrounded by cellular membrane. Some of the vacuoles were filled out with eosinophilic material (Figure 1B). The intercellular spaces in the irradiated melanomas were filled out with erythrocytes and other blood cells of which leukocytes looked deadly damaged. The blood and lymphatic capillaries were widened and had irregular form with features of erythrocyte-leukocyte stasis (Figure 1C). Hemorrhage was seen, created via perforation of necrotic walls of arterioles and venules. In some samples the walls of the blood vessels had turned into formless eosinophilic mass. There were clots consisting of fibrin and disintegrating leukocytes and erythrocytes within many blood vessels.

Neither tumor cells were found in histological sections got 1 hour after melanoma laser treatment, nor epidermal cells persisted within the laser field. Instead, collagen fibers due to coagulation necrosis were impregnated with single macrophages containing melanin (Figure 1D). The vessels both of dermis and subcutaneous fatty tissue were widened and hemorrhages as well as erythrocyte-leukocyte stasis were seen everywhere. The sebaceous and sweat glands and hair follicles were in a state of necrobiosis. There was insignificant infiltration of small lymphocytes and neutrophils on the periphery of skin appendices and vessels. Atrophy and disorganization of the texture due to papillary layer fibrosis were typical for the adjacent tissues.

One day after the laser application there was no intact epidermis in the treated area and collagen fibers and skin appendices were in the state of coagulation necrosis. The vessels were widened and overfilled with blood and there were fresh thrombi in some of them. Minor infiltration with small lymphocytes and neutrophils was found in the perivascular areas. In one case, foci of dystrophic tumor cells were revealed within necrotic masses and edema whereas the adjacent epidermis was atrophic and the dermis layers were indistinguishable.

Two days after the laser irradiation coagulation necrosis of the epidermis, dermis and skin appendices accompanied by hemorrhagic impregnation of the damaged tissue and hemorrhages made up the most typical microscopic picture. The vessels were overfilled with blood, some of them had wall rupture. There was moderate lymphocytic infiltration along the vessels.

Three days after the laser treatment coagulation necrosis covering entirely all skin layers and partly the subcutaneous fatty tissue was apparent in tissue specimens. Newly formed thrombi were seen in the vessels of the subcutaneous fatty tissue as well as infiltration of the deep layer of dermis and subcutaneous fatty tissue (mainly around the vessels and skin appendices) by

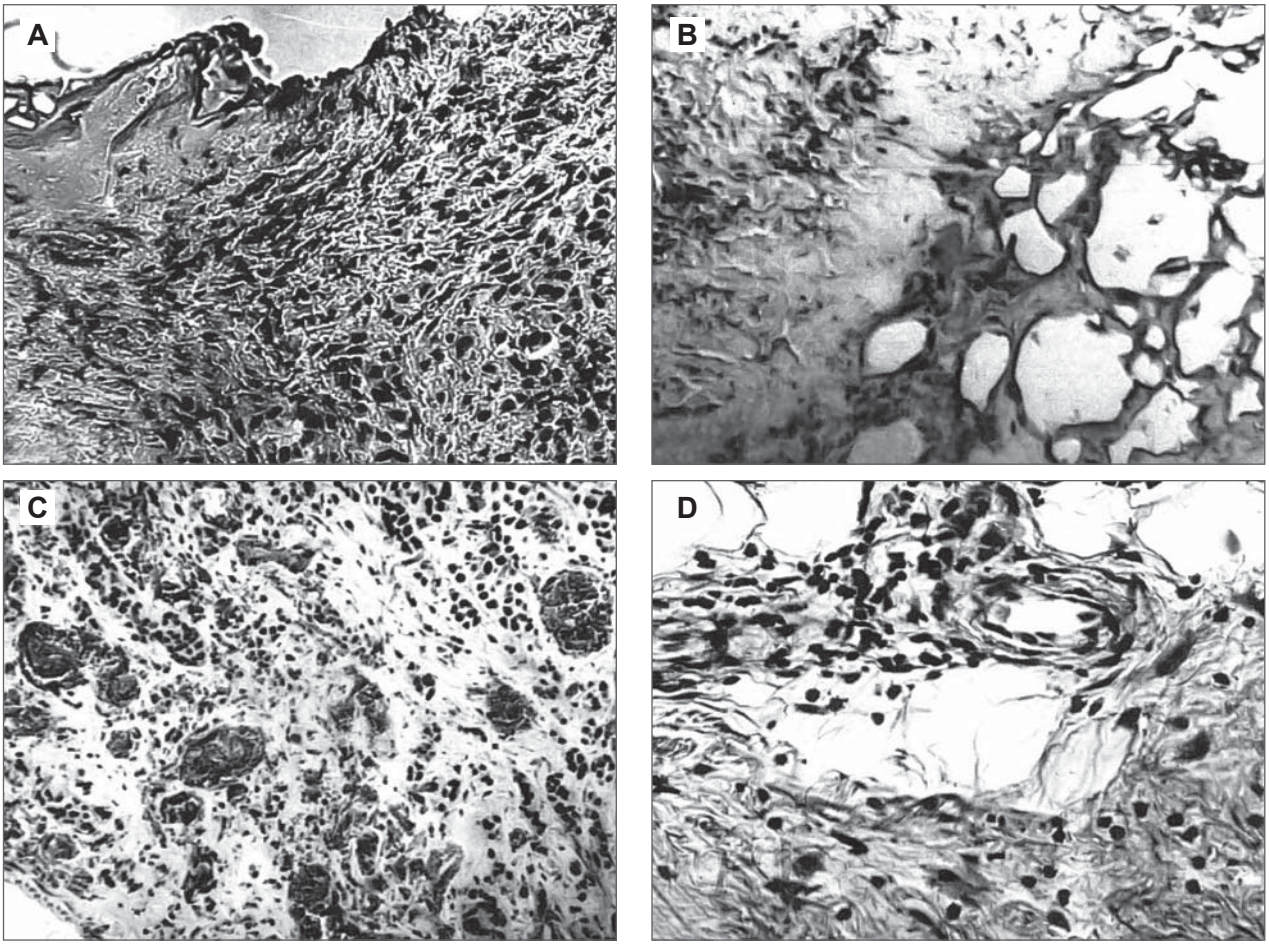

Figure 1. Photomicrographs of changes of cutaneous melanoma irradiated with pulsed Nd laser. **A:** Necrosis and dystrophic changes of tumor cells (H&E × 150). **B:** Tumor tissue loss of cell structure and bubble formation (H&E × 57). **C:** Widened blood and lymphatic vessels, and stasis (H&E × 150). **D:** Necrotic collagenous dermal fibers (H&E × 200).

small lymphocytes and neutrophils. In one case melanoma cells with dystrophic changes were observed in the scab.

Cytologic analysis of smears taken from the wounds revealed lymphocytes, macrophages, histiocytes and single nuclei but viable tumor cells.

Histological study of specimens of the epithelium-covered wounds two months after laser irradiation showed focal lymphoid infiltrates located mainly around the vessels (Figure 2). After 4 and 6 months, fibrosis with focal lymphoid infiltration and single giant cells were the only distinctive findings in the formed scars without tumor cells found in any of the sections.

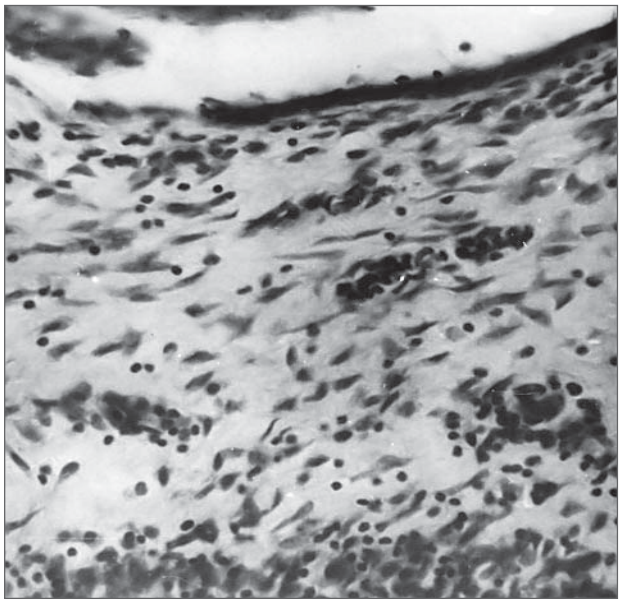

Figure 2. Photomicrograph of the scar 2 months after pulsed Nd laser irradiation of cutaneous melanoma of the left shoulder. Lymphoid infiltrates located predominantly around vessels. No tumor cells visible (H&E × 150).

Discussion

Laser irradiation causes acute destruction by means of thermal, hydrodynamic and photochemical effects of the laser beam, generating coagulation, evaporation and destruction of the tumor. Due to the very short

duration of the impulse and its high energy density the resulting tissue destruction has some unique peculiarities that discriminate those from ordinary thermal damage [6,12]. The possibility to create an entirely strictly local application and precise separation from the neighboring tissues are features of laser therapy. What is more, laser irradiation causes immediate thrombosis, stasis and coagulation of the blood and lymphatic vessels at the time of application [6], thus diminishing the probability of tumor cells' dissemination. The energy density of the laser impulse used in this study for cutaneous melanoma has induced complete destruction of the skin texture accompanied by necrosis of dermis and the superficial layers of the subcutaneous fatty tissue. However, in 2 cases dystrophically changed melanoma cells still persisted within the necrotic tissue after laser treatment. Comparable complexes of dystrophic tumor cells within the necrotic zone were present after Nd laser beam application in experimental melanoma Garding-Passey subcutaneously transplanted in mice [9]. These cells died with the lapse of time and the lapse was longer for cells with low density of melanin. This seems to reflect an uneven absorption of the laser energy density by melanoma cells depending on their melanin concentration.

It should be stressed that laser hit induces a prominent flow of immunocompetent cells like lymphocytes, macrophages and histiocytes into the affected areas what may be considered as a gain of local immunity [13].

The influence of the pulsed Nd laser irradiation upon the increase of the total quantity of vacant stroma cells from the epicenter to the periphery and their reproportioning toward an increase in the number of lymphocytes, macrophages and leukocytes was observed also in the B_{16} experimental melanoma [14]. The existence of prominent lymphoid and macrophage infiltration has been also reported in human skin irradiated with Nd laser for tattoo ablation [8]. The intensification of macrophage and histiocytic infiltration was also observed in intracutaneous melanoma metastases treated by continuous CO_2 laser [7], whereas after ordinary thermal burn the leukocytes infiltration prevailed [12]. In view of these data the low rate of melanoma recurrence after treatment with pulsed Nd laser [1-3] may be partly attributed to local immune reaction in response to acute photothermal damage of tumor and normal tissues by laser energy [13,15].

Conclusion

Irradiation of slightly elevated cutaneous melanoma by high-energy pulsed Nd laser produces coagulation necrosis of the tumor, dermis with the skin appendices and the superficial layers of the subcutaneous fatty tissue. The Nd laser energy induces acute photothermal damage of melanoma tissue, which differs from usual thermal lesions. Laser irradiation gives rise to immunocompetent cells infiltration of the affected area and this may be considered as an increase of local cellular immunity.

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