EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Role of Laser Energy Density for Photodynamic Therapy of Radiation Injuries of the Skin

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The role of different laser energy densities used in photodynamic therapy in the reparation of radiation ulcers caused by X-ray exposure in a dose of 80 Gy was studied. Tissue reparation manifested differently at different laser energy densities. After photodynamic therapy at energy density of 0.1 J/cm^2 the rate of healing was notably higher during early periods. After exposure at 5 and 40 J/cm^2 acceleration of ulcer healing was observed 14 and more days after the treatment, indicating the emergence of another mechanism of photodynamic therapy effect. Laser energy density of 5 J/cm^2 was the most effective for maximally complete healing of radiation ulcers.

Key Words: *photodynamic therapy; laser; radiation ulcers*

The processes of reparation of all skin injuries conform to the same laws. However, the type and severity of injury are essential for the reparation process. Healing of radiation ulcers depends on changes in biological characteristics of the damaged tissues, dysfunctions of body systems, and ulcer infection. These factors can delay cicatrization and led to the formation of stubborn unhealing ulcers. Therefore, the treatment should be complex. One of the main tasks of local therapy is restoration and stimulation of natural mechanisms of reparation. It was demonstrated that laser therapy activates growth factors (specific cytokines), stimulates enzyme production, promotes differentiation, proliferation, and migration of cells, *e.g.* fibroblasts containing basic fibroblast growth factor, an important regulator of angiogenesis [1,2].

Laser exposure as a monotherapy does not always produce stable positive results, and the problem of optimization of this treatment modality remains important. A possible direction is photodynamic therapy (PDT). Since biological effects directly depend on laser energy, the energy of PDT should be sufficient for biomodulating effect, but causing no tissue destruction. However, local reactions in the presence of pathological changes in the body can be inadequate to laser energies used in other situations.

We studied the role of laser energy density in PDT of radiation injuries to the skin in the presence of reactive (destructive degenerative) changes in the peripheral blood and analyzed the signs of hemopoiesis activation.

MATERIALS AND METHODS

The study was carried out on 60 female Wistar rats (170-200 g). Ulcers were induced by local X-ray exposure (80 Gy) of the mid-third of the back. The exposure conditions were as follows: 15 A current, 40 W voltage, 0.12 cm aluminum filter; 2×2 cm irradiated area, 50 sec exposure.

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The hair was thoroughly removed before the study. For immobilization during the exposure, the animals were intraperitoneally injected with 2.5% sodium thiopental (0.15 ml/100 g). Radiation ulcers formed 2 weeks after X-ray exposure. PDT was carried out during this period. Chlorine E6 0.6% gel served as the photosensitizer; the gel was applied as a thin layer (~1 mm) onto the ulcer surface. The rats were placed into individual containers for 2 h, after which the ulcer with a small portion of the adjacent tissue was exposed to laser (Lameda device). Laser energy densities were as follows: 0.1 J/cm^2 (group 1), 2 J/cm^2 (group 2), 5 J/cm^2 (group 3), and 40 J/cm^2 (group 4). Control group (5) consisted of untreated animals. PDT effect was evaluated by the coefficient of ulcer healing rate and duration of complete reparation. For estimating the coefficient, ulcer surface area (mm^2) was measured at different terms of healing: initial (before ulcer therapy; S_0) and on days 7, 14, 21, and 28 after exposure (S_t). Healing rate coefficient (C) was estimated by the formula:

$$C = \frac{S_0 - S_t}{S_0 \times t} \times 100\%,$$

where t is the day of measurement.

For evaluation of the general status of animals with radiation ulcers, the blood was collected from the caudal vein before and during therapy. Blood smears were dried in air and stained after Pappenheim (two-moment staining); peripheral blood cell composition and morphology (cell shape and size, presence of atypical cells, intensity of nucleus and cytoplasm staining, cell incorporations, *etc.*) were studied under a light microscope and differential blood count was estimated.

Bacterial contamination of ulcers was evaluated in control and experimental groups before and 2 weeks after PDT. The procedure was as follows: a smear was taken from the wound with a sterile tampon and the material was inoculated onto a Petri dish with nutrient medium. Bacterial contamination was evaluated after 24-h incubation.

The results were processed using Student's *t* test.

RESULTS

Despite local character of X-ray exposure and the use of lead shield protecting the animal, changes in the peripheral blood developed.

In all animals with radiation ulcers we observed destructive and degenerative changes characteristic of radiation reaction: vacuolation and pyknosis of the nuclei, cytolysis, fragmentation, chromatinolysis of neutrophils and lymphocytes; toxic granulation, anisocytosis (increased count of large cells and hypersegmentation of neutrophils); enhanced cell degradation, appearance of atypical cells, mainly atypical lymphocytes (Ryder forms), cells with nucleus deformation (brain-like distribution of chromatin), binucleated forms, and appearance of atypical cells in the blood.

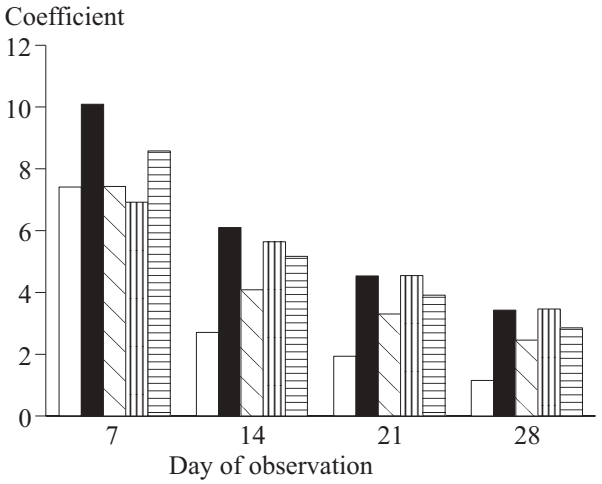

Fig. 1. Time course of radiation ulcer healing (healing coefficient) after PDT at different laser energy densities. Light bars: control; dark bars: 0.1 J/cm^2; cross-hatched bars: 2 J/cm^2, vertically hatched bars: 5 J/cm^2; horizontal hatching: 40 J/cm^2.

Inflammatory process in the ulcer and its infection were identified by changes in the quantitative and qualitative composition of peripheral blood cells: increased leukocyte count, appearance of immature forms, cells with nucleus deformation, atypical cells, *etc.*

Along with clear-cut destructive degenerative changes in blood cells, signs of hemopoiesis activation were noted, as was seen from moderate "left shift" in differential blood count (most pronounced for lymphocytes). Blast cells (lymphoblasts) appeared, as well as precursor cells (prolymphocytes, large lymphoid cells, large lymphoreticular cells), normally not observed in the peripheral blood.

Studies of bacterial contamination of radiation ulcer before therapy showed numerous colonies of *S. epidermidis* and in some animals *E. coli*.

This was the picture before PDT. Laser PDT at energy density of 0.1 J/cm^2 resulted in a statistically significant ($p<0.001$) acceleration of radiation ulcer healing during the first 7 days in comparison with the control. This rapid reparation was observed during all periods of observation (Fig. 1). The mean duration of complete healing of the ulcer in the group was 27±6 days (Fig. 2). Suppuration under

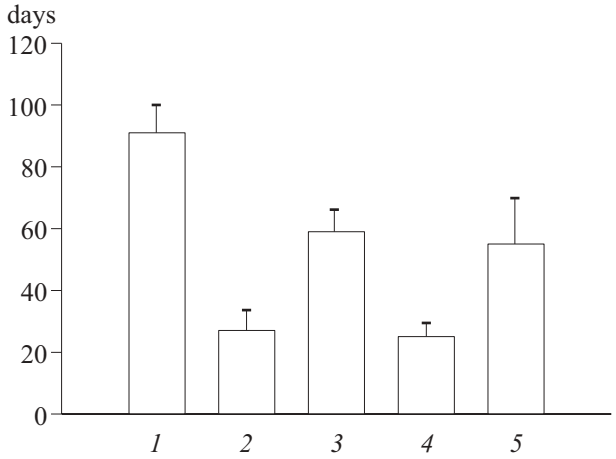

Fig. 2. Periods of complete healing of radiation ulcers (days) after PDT at different laser energy density. *1*) control; *2*) 0.1 J/cm^2; *3*) 2 J/cm^2; *4*) 5 J/cm^2; *5*) 40 J/cm^2.

the crust was noted in 20% animals, while in 80% rats healing was uneventful, and complete epithelialization of the ulcer was observed on day 22±2.

The stimulatory effect of PDT (2 J/cm^2 laser energy density) on ulcer healing rate was less pronounced. However, in later periods, starting from day 14 after exposure, the rate of ulcer healing was somewhat higher in the majority of animals in comparison with the control. The period of complete ulcer healing was prolonged to 59±5 days. Suppuration of the ulcer was observed in 50% rats. In other animals the mean duration of complete healing was 40±9 days.

PDT by laser exposure at energy density of 5 J/cm^2 led to acceleration of ulcer reparation only starting from day 14. None of the animals developed complications (suppuration). The period of complete epithelialization was also shorter.

The time course of radiation ulcer healing after PDT with laser energy density of 40 J/cm^2 was virtually the same as after 5 J/cm^2 (Fig. 1). However, 40% animals developed ulcer suppuration at later terms, which prolonged the period of complete healing. In cases without complications the mean duration of healing was 25±2 days. Bacterial contamination of ulcers decreased by tens times in these animals in comparison with the control. Peripheral blood morphology returned to normal only by day 21 of the study, but differential blood count did not normalize in comparison with the control. A slight increase in the neutrophil count, slight decrease in lymphocyte count, and increased count of monocytes persisted.

Hence, despite impaired organism's defense because of X-ray exposure, infection and inflammatory process in radiation ulcer manifesting by morphological and quantitative changes in white blood cells PDT of radiation ulcer activated ulcer healing. The efficiency of ulcer reparation directly depended on laser energy density. At energy density of 0.1 J/cm^2 healing was accelerated as early as during the first 7 days and remained pronounced during all periods of observation. However, this did not guarantee acceleration of ulcer healing in all animals, because in 20% cases we observed suppuration which deteriorated the reparation conditions. Early effect of healing stimulation was presumably due to stimulation of local growth factors accelerating epithelialization. The stimulatory effect of PDT at laser energy density of 2 J/cm^2 was less pronounced.

The best results were observed in animals receiving PDT at laser energy density of 5 J/cm^2. Ulcers were rapidly covered with a solid crust, which most likely prevented infection; angiogenesis was activated. This was seen from acceleration of reparation not during early periods, but on day 14. Presumably, the mechanism of radiation ulcer reparation after PDT of 40 J/cm^2 energy was the same. An appreciable reduction of bacterial contamination of ulcer indicated reduction of infection. However, the periods of ulcer healing after exposure at this laser energy density can be prolonged because of possible suppression of fibrosis, and this does not guarantee protection from infection during delayed period after PDT. Our results are in general in line with the data obtained in PDT used for the treatment of ulcers of other origin [2].

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