ONCOLOGY

Experimental Validation of the Use of PACKS-trypsin for the Prevention of Radiation Injuries of the Skin

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The latest advances in nuclear physics, radiobiology, dosimetry, and topometry have broadened the prospects of radiotherapy and improved its efficacy (Dzhelepov, 1981; Napalkov, 1981; Bardychev and Pereslegin, 1985).

Improvement of radiotherapy is aimed at seeking methods permitting the high dose irradiation of tumors involving the least possible exposure of normal tissues. Data on the effective use of high-energy radiation in the treatment of cancer of various localizations permitting the creation of high local doses have accumulated over the last two decades. However, it is impossible to rule out or to significantly reduce the incidence of radiation injuries of the skin associated with radiotherapy, and thus the search for methods of protecting healthy tissues presents a pressing problem. The use of PACKS-trypsin (trypsin immobilized on a synthetic textile matrix), a preparation created at the Oncology Department of the N. A. Semashko Moscow Stomatological Institute, is one of the methods of skin protection from radiation injuries. Clinical trials have demonstrated its high efficacy in the treatment of radiation injuries resulting from the radiotherapy of malignant tumors.

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The aim of the present research was to prove the efficancy of PACKS-trypsin as a means of protecting of normal tissues in the radiotherapy of malignant tumors.

MATERIALS AND METHODS

Experiments were carried out with white outbred rats of both sexes weighing 160 to 180 g. The animals were divided into two groups, experimental and control. A 4×4 cm skin site in the lower third of the back was locally irradiated. The hair on this site was thoroughly clipped. Irradiation was carried out with an Agat-R device under the following physicotechnical conditions: sourse-surface distance 75 cm, irradiation time (T) 800 sec, single focal dose 5 Gy, total focal dose 60 Gy, irradiation periodicity 24 h. The animals were fixed in a special stand during irradiation. To prevent total body irradiation, a special protective lead screen with a 4×4 cm hole cut in it was positioned over the irradiated area. The preparation was applied to the irradiated skin site on the cloth premoistened with normal saline (0.9% NaCl solution). A similar cloth, but without trypsin, was applied to the control animals' skin. Bioclinical monitoring and functional and morphological studies were carried out to confirm the radioprotective effect of the agent. The oxygen tension in the skin (pO_2)

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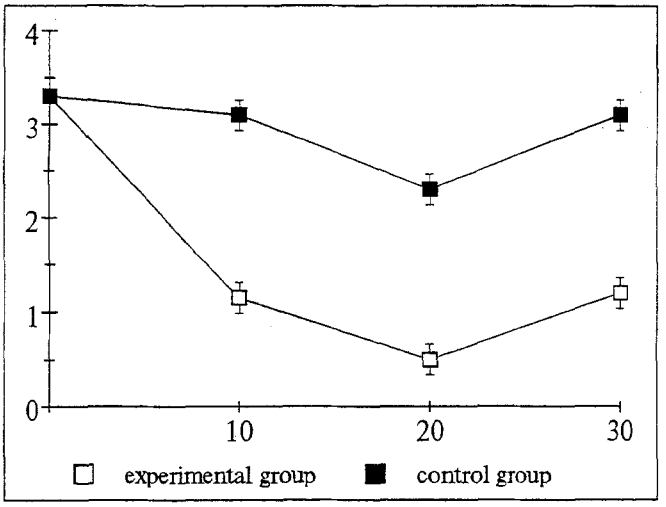

Fig. 1. Time course of pO_2 changes in the skin of experimental and control animals.

was chosen as the test of skin function. The measurements were carried out with a transcutaneous noninvasive TCM-2 monitor (Radiometer, Denmark) and pO_2 was measured with Clark E_{5242} closed-type transcutaneous transducer. The functional and morphological studies were carried out on days 10, 20, and 30 after the cessation of irradiation. The period of the investigation was chosen arbitrarily.

RESULTS

Erythema, stage one of radiation-induced epidermitis, was observed in animals of both groups, although the time of its appearance varied. In the control animals erythema developed after a total dose of 20-25 Gy on the average, that is, on days 5-6 of irradiation, while after a 30-35 Gy dose was administered dry symptoms of dry epidermitis developed.

In contrast, the experimental animals developed erythema after a dose of 35-40 Gy, that is, on the 7th-8th day. No stage of the dry epidermitis was observed in these animals.

Fur recovery at the irradiated skin sites was assessed visually. In experimental animals fur recovery started on days 7-8 after irradiation was discontinued and by the 12th-13th day the irradiated site was hardly discernible. That is why repeated hair removal was needed to measure the oxygen tension in the

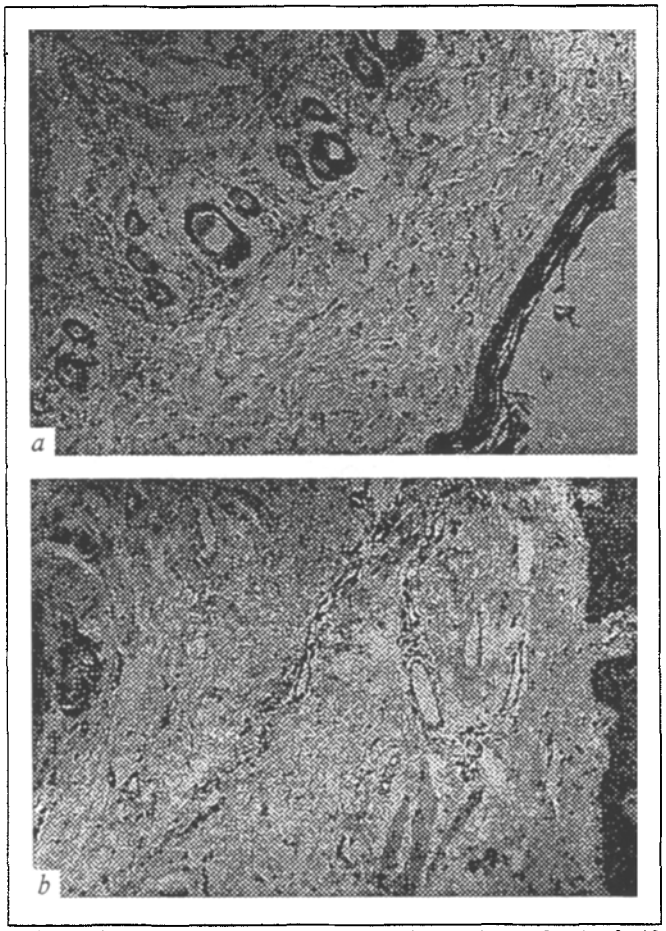

Fig. 2. Morphological changes in skin of experimental animals 10 days after irradiation. Here and in Figs. 3 and 4: *a*) experimental group; *b*) control group.

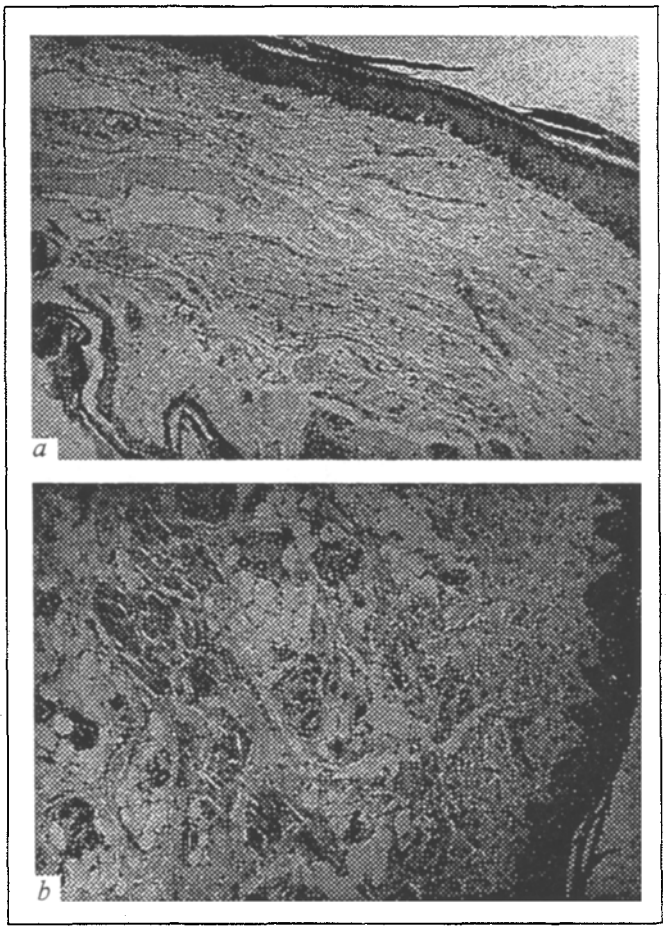

Fig. 3. Morphological changes in skin of animals 20 days after irradiation.

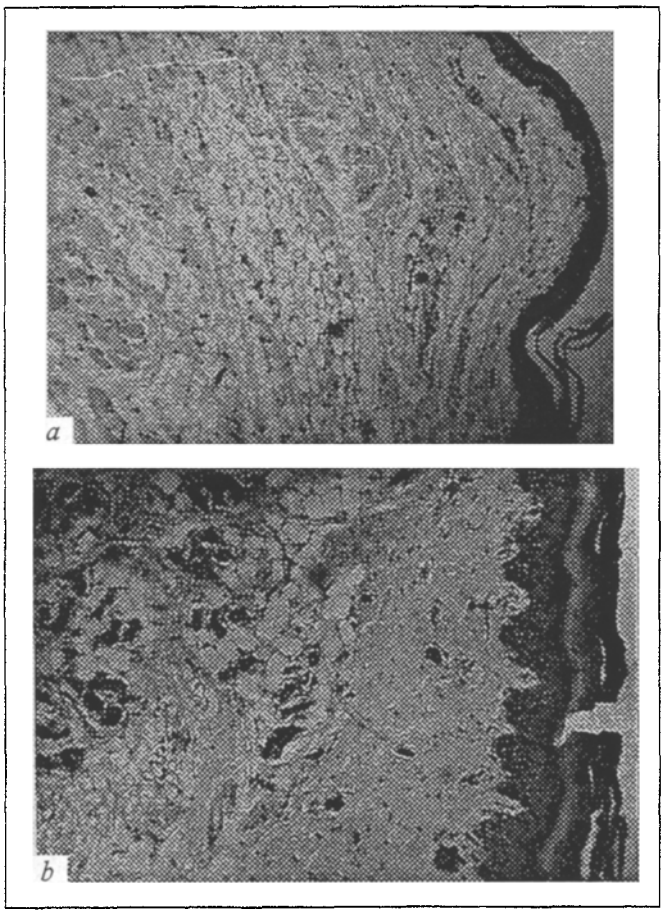

Fig. 4. Morphological changes in skin of animals 30 days after irradiation.

skin. In the controls fur recovery at the irradiated skin sites started on days 14-15 and was complete by the 27th-30th day after the end of irradiation.

The results of the pO_2 measurements are presented in Fig. 1. The scheme is based on the mean values calculated in the course of investigation. The data were statistically processed with an IBM PC XT computer. The initial pO_2 level in rat skin before irradiation was 3.27 ± 0.007 mm Hg ($t\geqslant2$, $p<0.05$). After a 10-day irradiation pO_2 was 3.21 ± 0.25 mm Hg in the experimental animals and 2.26 ± 0.3 in the controls ($t\geqslant4$, $p<0.01$ in both groups). After 20 days pO_2 was 2.40 ± 0.25 and 1.52 ± 0.10 mm Hg, respectively ($t\geqslant4$, $p<0.01$ in both groups) and after 30 days it was 3.01 ± 0.15 and 1.99 ± 0.15 mm Hg, respectively ($t\geqslant2$, $p<0.05$ and $t\geqslant4$, $p<0.01$, respectively). Hence, the measurement results are statistically reliable.

The experimental results indicate that the use of PACKS-trypsin as a local skin radioprotector is conducive to a more rapid recovery of tissue respiration and oxygen saturation of tissues, this leading in turn to a reduction of radiation injury of tissues.

Morphological changes in the experimental animals' skin were as follows (Fig 2, *a, b*). Ten days after irradiation was discontinued intensified keratinization of the epidermis, acanthosis and focal proliferation, and hair follicle atrophy were observed. The malpighian layer of the epithelium was almost intact. Cytoplasm vacuolation was observed in just solitary epithelial cells. Stasis phenomena were noted in the venules but not arterioles. The endotheliocyte and pericyte nuclei were hyperchromatic. The control animals developed within the same periods malpighian layer acanthosis, subepithelial connective tissue edema, and hair follicle atrophy; the endothelial nuclei were hyperchromatic. Venous stasis was observed in the derma proper.

Weakly expressed epithelial acanthosis was detected in the skin of animals administered PACKS-trypsin 20 days after irradiation course completion. Collagen fiber atrophy and fragmentation were observed (Fig. 3, *a, b*). The endotheliocytes were edematous in some microvessels, and the pericyte nuclei were hyperchromatic. The epitheliocyte nuclei were edematous, swelling in the vascular lumen and almost blocking it. The arteriolar, venular and capillary walls exhibited no apparent changes. In the controls the follicular structures showed virtually no signs of regeneration. Dermal edema and collagen fragmentation were observed, and the capillary and venular lumen narrowed. The endothelial nuclei were hyperchromatic. Along with flattened epitheliocytes cells with edematous cytoplasm and nucleus were found in arterioles, venules, and capillaries. The number of microvessels in which endotheliocytes obstructed the lumen was increased.

In the experimental animals no pathological changes were detected on day 30 of the follow-up, except for the thinned epidermal layer, signs of intensified keratinization of the surface layers, and collagen bundle thinning (Fig. 4, *a, b*). Mitoses were observed in the surface layer of the epidermis. In the control animals the changes were characterized by marked intensification of epidermal surface keratinization and dystrophy of some cells with cytoplasm vacuolation. Moderate acanthosis, hair follicle atrophy, and impaired follicle maturation were found. The endotheliocytes in the arterioles, venules, and capillaries were edematous and the microcirculatory bed vessels narrowed. Marked perivascular edema and collagen bundle thinning were observed in the derma; the connective tissue ground substance was edematous.