CHANGES IN MAST CELLS IN THE LOOSE SUBCUTANEOUS CONNECTIVE TISSUE AFTER LASER IRRADIATION

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Membrane preparations of the subcutaneous connective tissue of 150 mice after irradiation with a neodymium laser at different radiation densities were studied. With an increase in the radiation density (from 10 to 105 J/cm^2) the number of mast cells in the loose connective tissue increased. Laser irradiation of the skin led to intensive degranulation of the cytoplasm of the mast cells, which can be regarded as a manifestation of the active function of these cells in response to irradiation.

Changes in the mast cells in various pathological processes have been described frequently in the Soviet and non-Soviet literature [1, 2, 8, 9].

With the development of quantum electronics and the development of lasers, investigations into the action of laser radiation on biological objects have been described [4, 9, 10, 11].

However, no account could be found in the literature of the response of mast cells to laser irradiation.

The object of the present investigation was to study changes in the mast cells of subcutaneous connective tissue at various times after laser irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on 150 albino mice of both sexes. One area of the skin surface in the region of the spine was irradiated. A Soviet laser with glass as its working material, activated by neodymium ($\lambda = 1.06$, $t = 10^{-3}$) was used. The density of irradiation was 10.2, 48, and 105 J/cm^2. The hair was first shaved over an area of 5 × 2.5 cm for a laser beam diameter of 5 cm; an area of 2 × 2 cm for a beam diameter of 2 cm, and an area of 1.5 × 1.5 cm for a beam diameter of 1.4 cm; the irradiated areas of skin were marked with ink. The experimental animals were killed 2, 24, and 48 h and 5, 7, 10, 20, and 30 days after irradiation, together with an intact group of animals, by decapitation which did not affect the state of the mast cells.

The mast cells were studied in membrane preparations obtained by Yasvoin's method from the subcutaneous connective tissue in the irradiated area near the spine. The preparations were fixed in 10% neutral formalin solution and stained with toluidine blue at pH 3.25. In each series of experiments the number of loose connective tissue cells was counted. The number of mast cells was counted in the field of vision under the MBI-6 microscope with an enlargement of 250 ×. Statistical analysis of the results was carried out by I. A. Oivin's method.

EXPERIMENTAL RESULTS

A unique feature of these experiments was the investigation of the subcutaneous connective tissue after comparatively low densities of laser irradiation not producing thermal trauma to the animals' skin.

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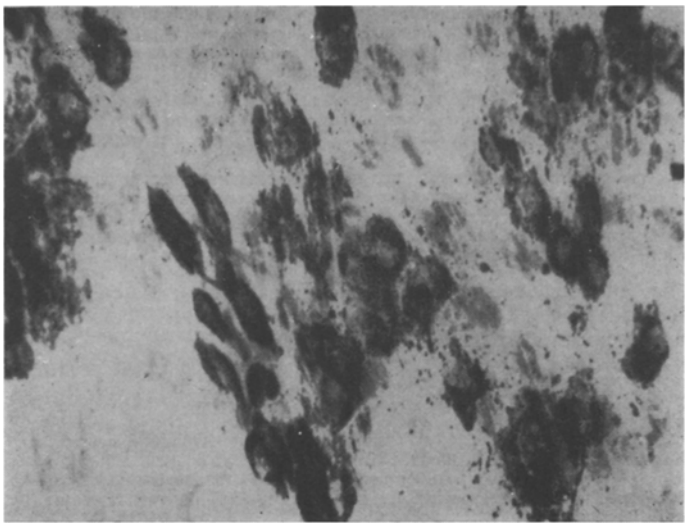

Fig. 1. Mast cells in loose subcutaneous connective tissue after laser irradiation of mouse skin. Toluidine blue, 500 ×.

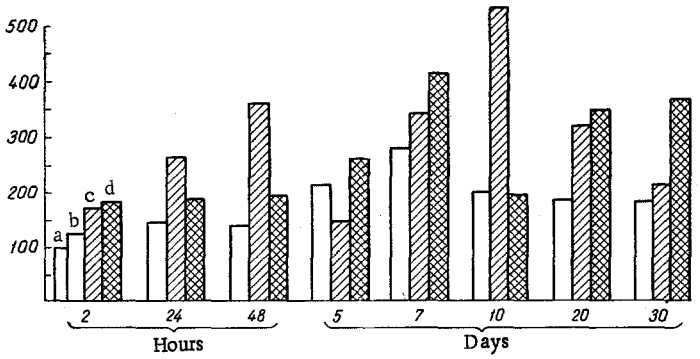

Fig. 2. Changes in number of mast cells in subcutaneous connective tissue of mice after laser irradiation: a) control; b) 10.2 J/cm^2; c) 48 J/cm^2; d) 105 J/cm^2. Abscissa, time of investigation; ordinate, number of cells (in %).

The results of microscopic examination of membranes taken from the mice at the site of laser irradiation of the skin with densities of 10.2, 48, and 105 J/cm^2 showed that the most typical, indeed stereotyped, expression of the changes taking place in the connective tissue was a diffuse macrophagal reaction which varied in intensity. In the presence of a macrophagal reaction in the connective tissue the total number of cells increased, and the quantitative ratio between the two chief functionally different groups of cells (fibroblasts and macrophages) altered. With all the doses of irradiation described morphological changes in the fibrous structures of the ground substance could be observed. Mast cells, as a special functional group of cells with, in the modern view, metabolic connections with the ground substance, were studied separately.

In mice irradiated with an unfocused laser beam with density 10.2 J/cm^2 the following morphological changes were observed in mast cells in the loose subcutaneous connective tissue taken from beneath the area of irradiated skin. The total number of mast cells 2 h after irradiation was increased by 24% ($P < 0.05$) compared with their number in the control animals. The mast cells under the microscope appeared to have more or less clearly defined borders: the nuclei were round in shape, indistinctly outlined, and blue in color. The cytoplasm of the mast cell was stippled with metachromatic granules. Some cells had discharged their granules into the surrounding connective tissue. The mast cells were arranged along the course of the blood vessels and also in the connective tissue itself (Fig. 1).

The number of mast cells in the loose connective tissue after laser irradiation at this density increased gradually, starting from soon after irradiation; on the 7th day their relative percentage was at its highest, 187% higher ($P < 0.001$) than in the control. After 10-20 days the number of mast cells was smaller than at the previous period after irradiation but was still 94% higher than the control ($P < 0.001$).

With an increase in the density of irradiation to 48 J/cm^2 the total number of mast cells in the loose subcutaneous connective tissue increased. In preparations obtained 2 h after irradiation most mast cells had a degranulated cytoplasm; cells of this type were four times more numerous than in the control ($P < 0.001$). Outside the cells the number of metachromatic granules varied: often the granules were clustered together and formed distinctive conglomerations in the connective tissue. After discharge of the granules from the cytoplasm the cell itself became less clear, and empty spaces and light vacuoles could be distinguished in it instead of metachromatic granules. The number of mast cells with well-marked degranulation was reduced 5-10 days after laser irradiation. At these times after irradiation mature mast cells were predominant. Their cytoplasm was filled with large metachromatic granules. The nuclei of the mast cells were clearly outlined and in some cases were masked by granules. A characteristic feature of the tissue mast cells at all times after irradiation was their irregular clustering together. The mast cells were arranged in groups or chains each containing 20 to 30 cells. However, solitary examples could also be seen. On the 10th day after irradiation the total number of mast cells reached the maximum for that dose of irradiation, almost $4^1/_2$ times greater than the control (440%, $P < 0.001$; Fig. 2). The relative proportions of the mast cells again varied 20-30 days after irradiation. The number of cells with degranulating cytoplasm increased again. Their number was two to three times greater than in the control.

With an increase in the density of irradiation (105 J/cm^2) the number of mast cells increased; they were arranged both along the blood vessels and at a distance from them. Specific granules accumulated in the cytoplasm of the mast cells, and the intensity of their metachromatic staining increased. The number of mast cells 2 h after irradiation was 81% greater ($P < 0.05$), and 7 days after irradiation four times greater (325%, $P < 0.02$) than in the control. Their number 20-30 days after irradiation was increased by almost three times above the control level, or by 276% ($P < 0.01$). At the same time (20-30 days) the number of cells with marked degranulation of their cytoplasm was increased. Their number was $1^1/_2$ times greater than the control.

It is worth noting that a similar increase in the number of mast cells in the connective tissue was observed by Shikhodyrov [5-7] in chronic radiation sickness.

LITERATURE CITED

1. L. A. Afrikanova, Arkh. Pat., No. 11, 42 (1962).
2. K. M. Danilova, Arkh. Pat., No. 1, 3 (1958).
3. K. K. Rudzit, Izvest. Akad. Nauk Latv. SSR, No. 1, 143 (1953).
4. V. N. Shevchenko and B. Ya. Gol'shmid, in: Carcinogenesis, Methods of Diagnosis,and Treatment of Tumors [in Russian], Kiev (1968), p. 97.
5. V. V. Shikhodyrov, Arkh. Pat., No. 9, 35 (1957).
6. V. V. Shikhodyrov, in: Abstracts of Proceedings of the Sixth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Khar'kov (1958), p. 700.
7. V. V. Shikhodyrov, in: The Role of Connective Tissue and the Blood System in Radiation Pathology [in Russian], Moscow (1970), p. 113.
8. C. L. Arvy et al., C. R. Acad. Sci. (Paris), 232 (1951).
9. F. Colzavara et al., Riv. Anat. Pat., 17, 274 (1960).
10. F. Helwig, Fed. Proc., 24, 83 (1965).
11. S. Laor et al., J. Path., 47, 643 (1965).