The Effect of Bombardment with Continuous Infrared Laser on the Ultrastructure and Proliferation of Ehrlich Carcinoma Cells

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Bombardment of Ehrlich carcinoma with infrared laser for 3-5 min daily during a 10-day period resulted in an almost 2-fold increase in the index of labeled nuclei without any increase in mitotic index and tumor mass. The differences between the index of labeled nuclei and mitotic index revealed by electron microscopy are due to pronounced changes in the nuclei: "squeezing" of chromatin through pores or ruptures in the nuclear membrane and its random location in the cytoplasm. The number of macrophages, neutrophils, and plasma cells in the tumor increases.

Key Words: *laser; Ehrlich carcinoma; ultrastructure; proliferation*

Although low-intensity laser irradiation, particularly in the infrared and red range, has been widely used to treat numerous pathological processes and states [1-5,9] and to stimulate the immune system in patients with tumors [7], its effect on tumor cells has not been studied in sufficient detail. Specifically, the effect of continuous low-intensity infrared (IR) laser on the ultrastructure and proliferation of tumor cells has not been investigated.

Our aim was to study the effect of repeated bombardment of low-intensity IR laser on the structure and proliferation of Ehrlich carcinoma cells.

MATERIALS AND METHODS

A total of 32 outbred albino mice were used. Irradiation (3 and 5 min once daily at 9 a.m.) was started on day 7 after tumor induction. The output of the arsenide-gallium laser (Aura) was 180-200 mW. The distance between tumor and laser was not more than 0.5 cm. Tumor morphology was studied prior to irradiation and on days 5 and 10 after it was begun. Control tumors were not irradiated. For the determination of proliferative activity ^{3}H-thymidine (18 μBq/g body weight) was injected intraperitoneally 1 h before decapitation.

Sections (4-5 μ thick) were covered with "M" emulsion, exposed for 1 month, and developed in an amidol developer. The sections were stained with Ehrlich hematoxylin, and the index of labeled nuclei (ILN) was determined. A minimum of 5000 nuclei were counted in each group, and at least 10,000 cells were counted upon morphometric determination of the mitotic index (MI). For electron microscopy the tissue was fixed with 2.5% glutaraldehyde and postfixed with 1% OsO_4 in phosphate buffer. Double-contrasted Epon-Araldite ultrathin sections were studied in a Hitachi-H600 electron microscope. Semithin sections were stained with Methylene Blue and fuchsin.

Data on tumor mass and morphology were processed by the methods of variational statistics using dedicated software.

RESULTS

On day 7 after tumor induction a round, dense formation about 0.5 cm in diameter was found in the inguinal region.

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The tumor consisted of oval cells with hyperchromatous nuclei varying in size and containing large nucleoli. Numerous pathological mitoses were seen; MI was 9.4%.

Electron microscopy showed that the cytoplasm of tumor cells contains numerous ribosomes, occasional profiles of granular endoplasmic reticulum, and mitochondria evenly distributed in the cytoplasm. Nuclei with deep invaginations, numerous pores, and large nucleoli were seen. Mitoses were clearly visible in semithin and ultrathin sections (Fig. 1, *a*, *b*).

By days 12 and 17 after induction, the tumor had increased in size but its structure remained unchanged. On day 17, necroses were observed in the center of the tumor; some mice had developed skin ulcers over the tumor.

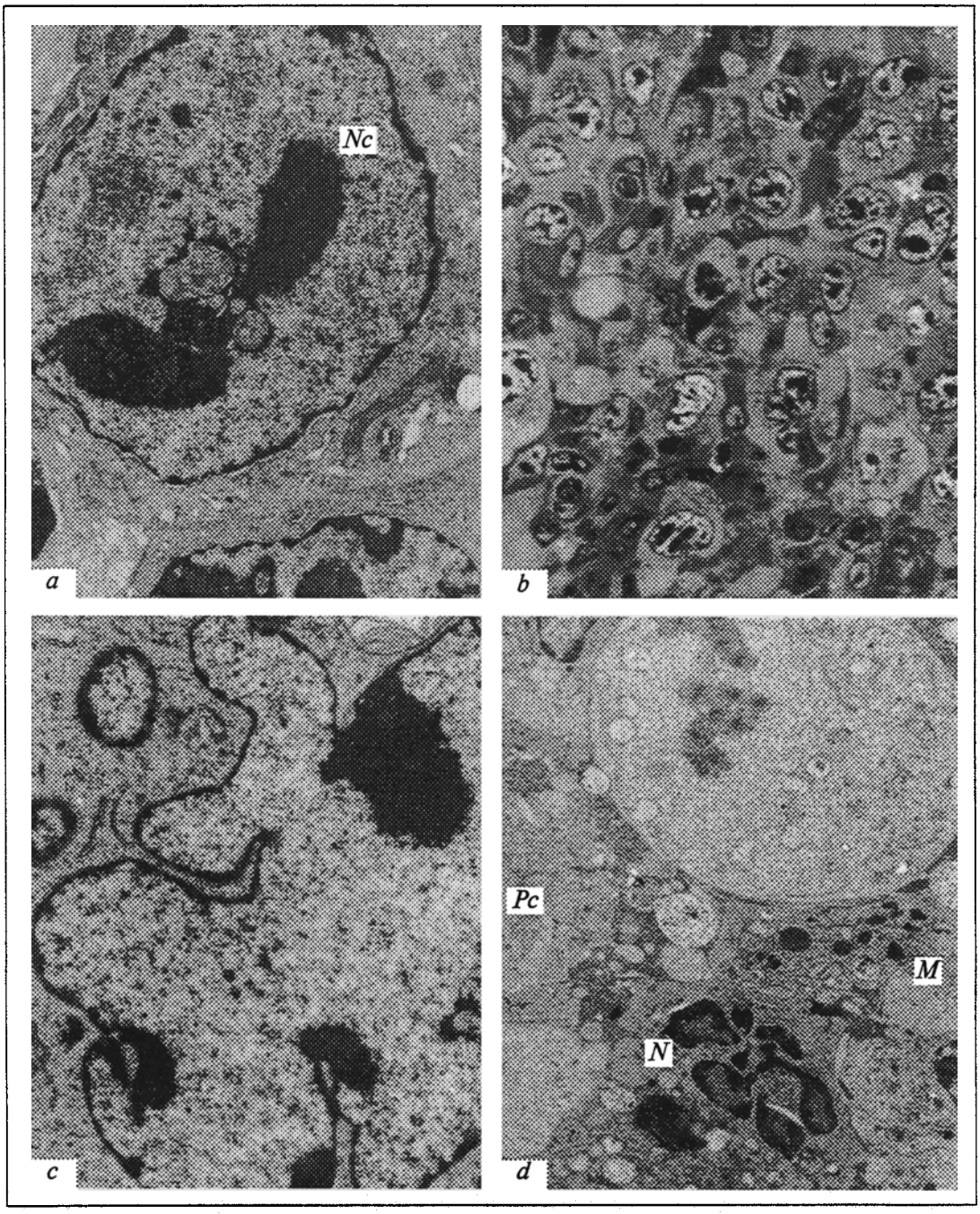

Fig. 1. Effect of IR laser on the ultrastructure of Ehrlich carcinoma cells. *a*) large nucleoli (*Nc*) and invaginations in the nucleus of a tumor cell on day 10 after tumor induction (control). Transmission electron microscopy (TEM), ×7500; *b*) polymorphism of tumor cells and their nuclei, mitoses, day 10 after induction (control). Semithin section, Methylene Blue–fuchsin, ×400. *c*) nucleus of a tumor cell with numerous invaginations after five 3–min irradiation sessions. TEM, ×7500; *d*) neutrophil (*N*), macrophage (*M*), and plasma cell (*Pc*) after ten 5–min irradiation sessions. TEM, ×3750.

After five sessions of irradiation, there were no substantial differences in the size and mass of tumors in control and experimental mice. The number of pathological mitoses in tumors increased but MI decreased. There were more cells with vacuolar and hydropic dystrophy. More invaginations of the nuclear membrane and nucleoplasm containing profiles of granular endoplasmic reticulum were seen (Fig. 1, *c*).

Substantial differences were revealed by light and electron microscopy and radioautography after ten sessions of laser therapy. The tumors irradiated for 5 min were smaller than those irradiated for 3 min, weighing 1 and 1.3 g, respectively (1.4 g in the control).

Radioautography showed that the incorporation of ^{3}H-thymidine in the nuclei of tumor cells varied considerably from group to group. In mice irradiated for 3 or 5 min during a 10-day period, ILN increased almost 2-fold (Fig. 2). However, this increase was not accompanied by a corresponding increase in MI and tumor mass. By contrast, in irradiated animals the tumor mass showed a tendency towards a decrease.

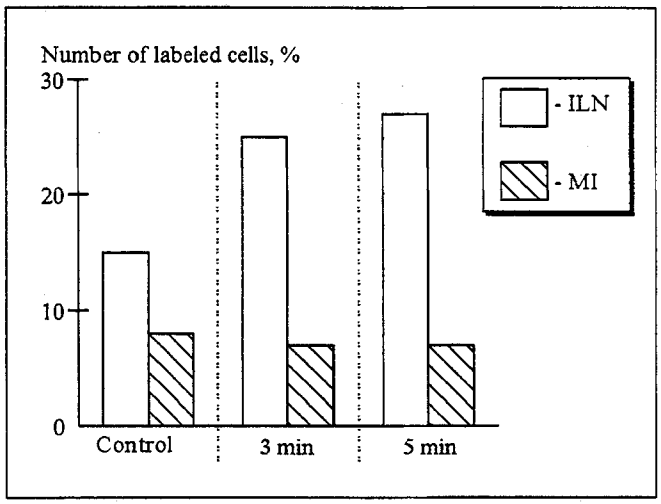

Fig. 2. Proliferative activity of tumor cells irradiated with IR laser (10 sessions).

The increase in ILN countered by a decrease in MI was an unusual phenomenon. Generally, there is a positive correlation between these indexes in all populations of proliferating cells [8].

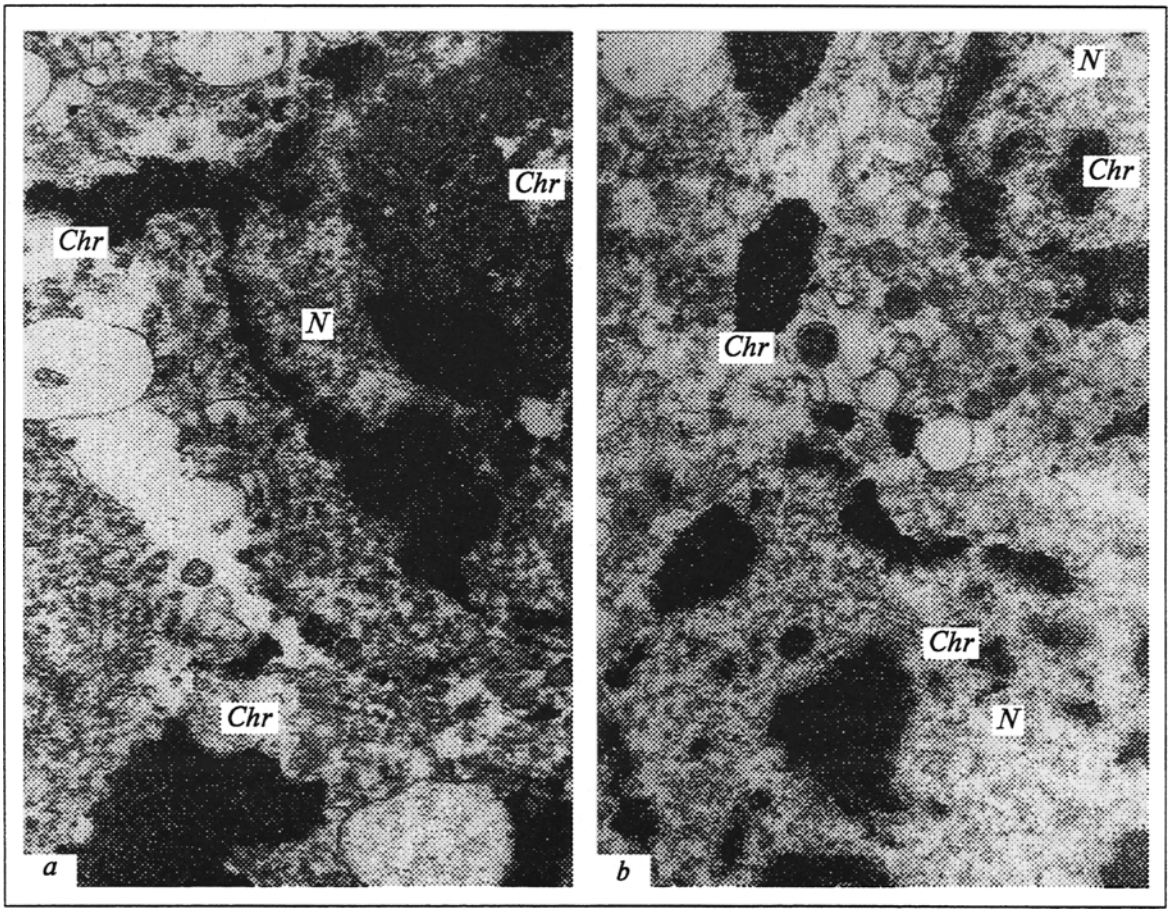

Fig. 3. Changes occurring in the nuclei of Ehrlich carcinoma cells irradiated with an IR laser. TEM, × 15,000. *a*) "squeezing" of chromatin (*Chr*) from a nucleus (*N*) with preserved integrity after ten 5-min sessions of irradiation; *b*) the same phenomenon with "melting" of the nuclear membrane.

The situation was clarified by electron microscopy. The ultrastructure of tumor cells was markedly changed, particularly in the group where the tumors were irradiated for 5 min. Swollen mitochondria with a transparent matrix and myelin structures were revealed. The profiles of granular endoplasmic reticulum were fragmentized. Changes were most pronounced in the nuclei: the nuclear membrane was disrupted, and the nucleoplasm content, the chromatin above all, was "squeezed out." In most cells where the integrity of the nuclear membrane was preserved, the chromatin was "squeezed" through nuclear pores (Fig. 3) and was distributed in the cytoplasm or in the intercellular space.

These changes differ from those occurring during apoptosis, when cell death is accompanied by margination of chromatin and condensation and fragmentation of the cytoplasm with the formation of apoptotic bodies [6].

In addition to these changes, the number of neutrophils, plasma cells, and macrophages in the tumor increased (Fig. 1, *d*). Thus, bombardment of Ehrlich carcinoma cells with a continuous 180-200 mW IR laser markedly raises ILN without inducing any change in MI, since it damages the nuclei and causes the chromatin to be "squeezed" through nuclear pores or ruptures of the nuclear membrane.

The drop in the rate of tumor growth after irradiation with a continuous IR laser is presumably based on this phenomenon.

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