Effect of Laser Radiation Proliferative Activity of Cultured Cells

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Using morphometry and radioautography methods, it is found that a single laser irradiation (0.5 and 0.05 J/cm²) of cultured human fibroblasts stimulates their proliferation *in vitro*. The effect of the laser is realized via intensification of DNA synthesis.

Key Words: *proliferation; tissue culture; stimulation; laser*

In recent years lasers have found a wide therapeutic application [5,7-9]. However, the mechanisms of laser-induced biostimulation remain obscure [4], so that the clinical application of lasers is often empirical. The practical significance of laser therapy can be assessed after analysis of experimental and clinical observations geared toward a better understanding of the changes occurring in human and animal cells and tissues irradiated with laser. It has been shown that laser light can induce considerable structural alterations in cells and tissues [3], thus affecting cell proliferation [1,2] and differentiation.

In this study the proliferative activity of human fibroblasts irradiated with varied doses of laser emission was evaluated by the method of radioautography.

MATERIALS AND METHODS

Cultures of human fibroblasts were used. A primary cell culture was obtained routinely [6]. Cells were irradiated after the 3rd-4th subculturing on day 2 after passage. Cells were seeded on plastic dishes (Costar) at a density of 2×10^5/cm² and cultured in Eagle's medium with conventional additives before and after treatment with laser light (a single pulse, 890 nm, Gelios or Lazur' apparatus). Energy exposure was 0.05 J/cm² (group I) or 0.5 J/cm² (group II). Intact cell cultures served as a control (group III).

Morphological examination was performed immediately before laser irradiation and 1, 3, and 5 days after it. Proliferative activity was assessed by counting cells under an Opton inverted microscope (magnification 250). Incubation with ^{3}H-thymidine was carried out for 1 h, after which the cells were washed and fixed. The index of labeled cells was determined at all stages of the observation. Cell cultures were stained with toluidine blue. The data were analyzed using standard statistical methods.

RESULTS

The mean cell density before laser irradiation was 18±5.99 per square unit. The cells were of spindle or stellate shape; the nuclei with one or two nucleoli were clearly seen. The cytoplasm was compact, homogeneous, without vacuoles. These cultures contained not more than 3% of ^{3}H-thymidine-labeled cells (Fig. 1).

Morphological differences between the control and laser-treated cultures were observed on day 1

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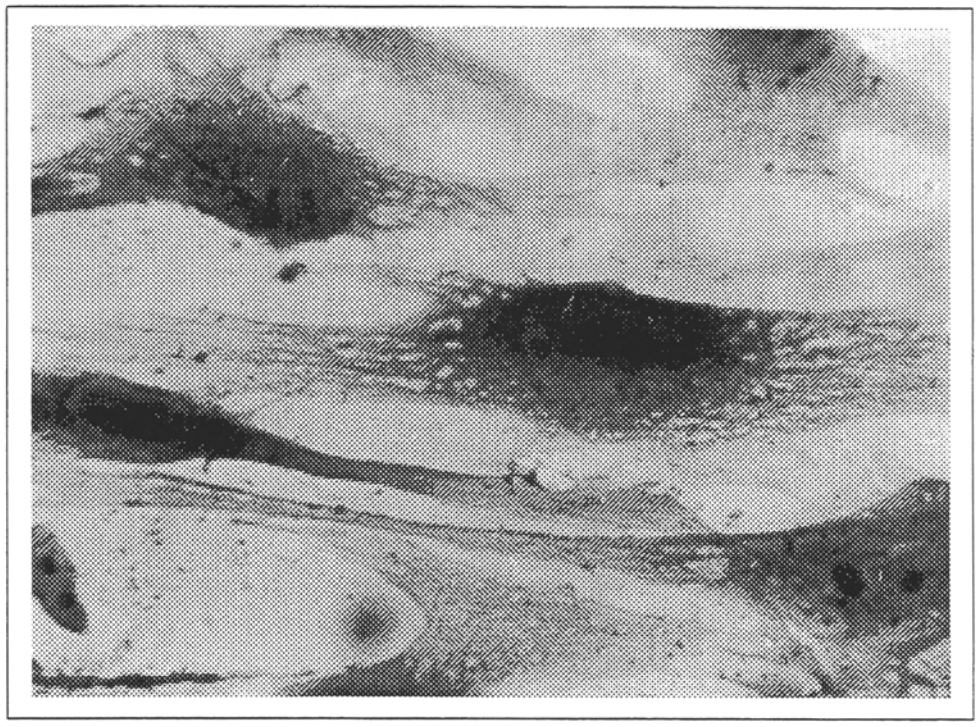

Fig. 1. Intact culture of human fibroblasts. The cells do not incorporate ^3H-thymidine. Here and in Fig. 2: toluidine blue staining. ×1000.

after irradiation. Cell density and the number of labeled cells in control cultures remained virtually unchanged: 18±7.62 and 2%. There were no changes in the cell morphology.

In laser-treated cultures, cell density and the number of labeled cells ([^3H]-thymidine incorporation in DNA) increased considerably (Fig. 2).

In group I cultures (irradiation dose 0.05 J/cm^2) these changes were more pronounced than in group II cultures (0.5 J/cm^2). In group I cultures cell density increased to 52±4.49 (by 289%) and 12% of cells incorporated the label (vs. 2% in the control cultures); in group II cultures these parameters were 29±0.9 (an increment of 139% and 10%, respectively.

On day 1 after irradiation the number of dividing cells increased. Nuclei and nucleoli in irradiated cells were larger than in intact cultured

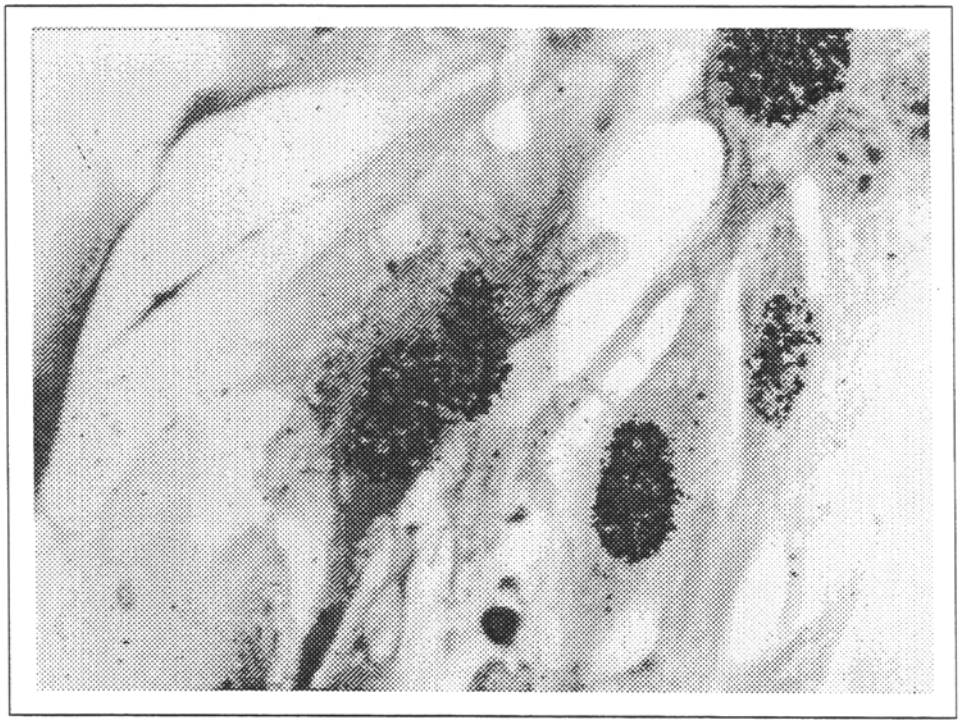

Fig. 2. Culture of human fibroblasts on day 1 after laser irradiation (0.05 J/cm^2). Numerous cells labeled with ^3H-thymidine are seen.

TABLE 1. Effects of Laser Radiation on ^3H-Thymidine Incorporation in Cultured Human Fibroblasts (%)

Group	Day of observation		
	1	3	5
I (0.05 J/cm²)	12	32	4
II (0.5 J/cm²)	9	26	2.6
III (control)	2	11	9

TABLE 2. Effects of Laser Irradiation on Cell Density in Human Fibroblast Cultures (Number of Cells in the Field of View)

Group	Day of observation		
	1	3	5
I (0.05 J/cm²)	52±4.49	54.9±5.7	72±7.2
II (0.5 J/cm²)	29±0.9	59.4±6.5	78.4±5.3
III (control)	18±7.6	30±4.1	38.5±6.5

cells; numerous cells in irradiated cultures contained 3-4 nucleoli.

On day 3 after laser irradiation, control cultures had growth foci. Cell density in these cultures significantly increased (30±4.1), and 11% of cells had incorporated the label. Both irradiated cultures reached confluence. Although the differences in cell density and in the number of labeled cells between the control and experimental groups were preserved, the cell density in group I cultures was close that in and group II cultures, this being accompanied by a leveling of the differences in the number of labeled cells in these groups (Table 1).

On day 5 there was no difference in cell density and number of labeled cells in the irradiated cell cultures. Cell density in the control group was much lower, although the number of labeled cells was high (Table 2).

These findings show that even a single irradiation of fibroblast cultures with laser light stimulates the proliferative activity of these cells. This holds true for both doses: 0.5 and 0.05 J/cm². The stimulatory effect of the lower dose (0.05 J/cm²) is realized 1 day after irradiation; it develops rapidly and is prolonged (5 days).

The effect of the higher dose (0.5 J/cm²) is realized on day 1 after irradiation, but it does not develop as rapidly; however, on day 5 it cannot be distinguished from that produced by the lower dose.

Radioautography has shown that the effect of laser irradiation is achieved through changes in DNA synthesis. This is documented by the higher incorporation of ^3H-thymidine in the cells during the first stages of observation. The reduction in the intensity of synthetic processes on day 5 after irradiation is explained by contact inhibition due to the formation of a confluent monolayer. The dynamics of cell density in irradiated cultures indicates that lasers affect both the rate and intensity of cell proliferation.

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