METHODS

Effect of Monochromatic Red and Near-Infrared Light on the Adhesive Properties of the Cell Membrane: Dependence on Wavelength

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Changes in the adhesive properties of the cell membrane after irradiation of HeLa cells with monochromatic visible and near-infrared radiation (λ=580-860 nm, i=1.3 W/m^2, t=40 sec, D=52 J/m^2) are assessed as the number of adherent cells. Three spectral regions (600-625 nm, 645-700 nm, and 720-860 nm) with the maximums near wavelengths 620, 680, 750, and 825 nm are identified, where the adhesive properties of the cell membrane are observed to be enhanced.

Key Words: *cell membrane; low-power laser therapy; surface-adhesive properties; effective spectrum*

Many experimental and clinical studies have been performed on the interaction between the low-intensity monochromatic light from laser or other sources and cells and tissues [3,6]. These studies have provided a basis for laser therapy. However, the question as to the optimal wavelength of laser therapeutic devices is still unclear. A great number of different sources of radiation either in the red (He-Ne laser, λ=632.8 nm) or in the near-infrared (IR) spectral regions (laser sources and light emission diodes) are used in both laboratory and clinical practice. The optimal wavelength can be determined on the basis of the effective spectrum (biological effect as a function of the wavelength used for irradiation). Clinical studies of effective spectra are still lacking. Effective spectra at the cellular level are available for the multiplication rate of microorganisms [2,4] and for the rate of DNA and RNA synthesis in cells in both the exponential [7] and the stationary [8] growth stage.

We showed previously that after irradiation with light at l=632.8 nm the adhesive properties of HeLa cells alter, depending on the times postirradiation [1].

The objective of the present study was to determine the adhesive properties of the cell membrane as a function of the wavelength used for irradiation.

MATERIALS AND METHODS

HeLa cells were cultured in scintillation vials in medium 199 containing 10% bovine serum and antibiotics (penicillin and streptomycin, 100 U/ml each). Seventy-two hours after seeding, the cell monolayer was removed and a cell suspension was prepared under mild conditions, as described previously [1]. Such a method of preparing suspension results in minor damage to the cells, and the suspension virtually consists of separate cells

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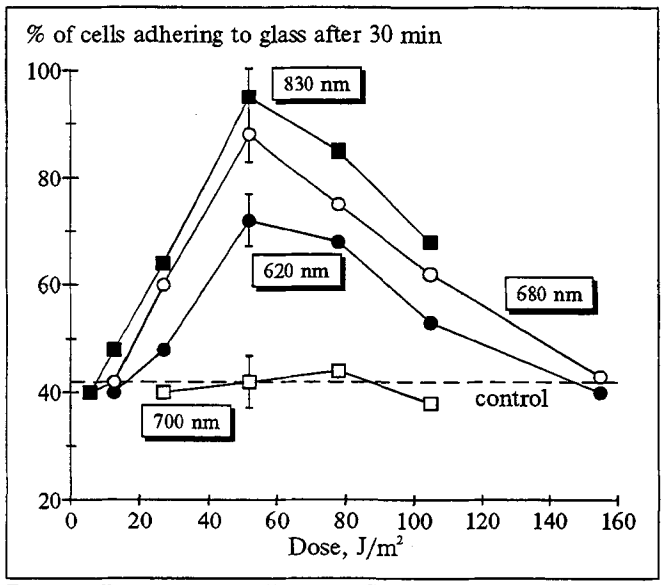

Fig. 1. Percentage of adherent cells as a function of radiation dose for different wavelengths.

(89.6%±0.9%). This suspension was used for preparing a cell suspension in medium 199 (supplemented with 10% serum), which was placed in a special glass cuvette for irradiation. The volume of the cuvette was 130 μl (inner diameter 0.7 mm, thickness of suspension layer 4 mm, cell count 85,000). The cuvette was filled right to the top in order to avoid the formation of a meniscus. Monochromatic radiation (an EKsEl-250 xenon lamp with a power of 250 W served as a light source) was produced using a monochromator (spectral range 540-1050 nm) designed by A. M. Lifshits of the Institute of Spectroscopy. The monochromator worked in autocollimation regime. The spectral width of output radiation was 10 nm. The monochromatic radiation was led from the lower part of the aperture with a multifiber light guide and directed at a right angle to the irradiated suspension. The area of the light spot covered the area of the suspension irradiated (0.38 cm²). The distance between the tip of the light guide and the bottom of the cuvette was 24 mm. Within the region from 580 to 860 nm the output power was 0.050 mW (after passing through the suspension 0.035 mW) for all spectral bands. The intensity was 1.3 W/m². After irradiation the cuvette was placed in an incubator for 30 min (this period included the duration of irradiation). The number of cells adhering to the bottom of the cuvette during 30 min served as the index of changes in the adhesive properties of the cell membrane. For determination of the number of adherent cells the suspension was decanted, the cuvette was rinsed with Hanks solution, and adherent cells were removed with 0.02% Versene solution and counted in a Goryaev chamber. The data are presented as the mean of 10 measurements for each point.

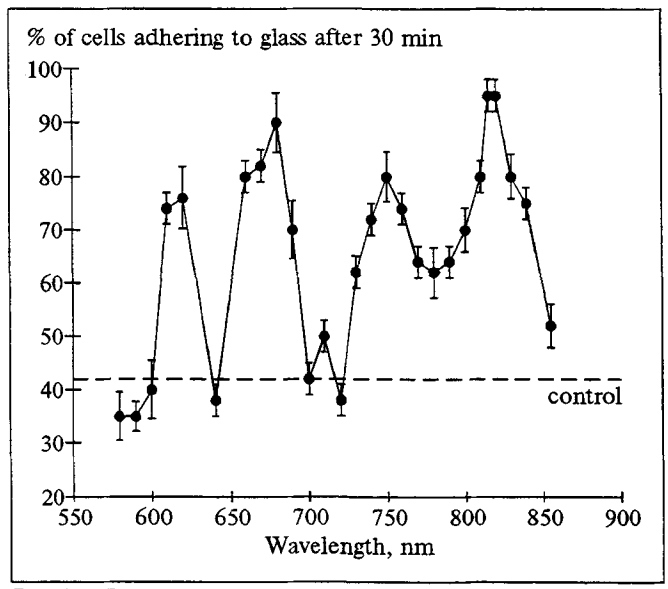

Fig. 2. Percentage of adherent cells as a function of radiation wavelength (effective spectrum). Dose 52 J/m², time of irradiation 40 sec, intensity 1.3 W/m².

RESULTS

Under the conditions of our experiment 42±3% of the total number of cells (85,000) adhered to glass in nonirradiated cuvettes. Irradiation increased the number of cells adhering to glass. In Fig. 1 the percentage of adherent cells is presented as a function of the dose of radiation for several wavelengths. As is seen from the figure, the maximum increase of the fraction of adherent cells was observed in all cases for the dose of 52 J/m², although the percentage of adherent cells (i.e., the intensity of the effect) was different at different wavelengths. A dose of 52 J/m² was then used in measurements of the effective spectrum.

The effective spectrum for changes in the adhesive properties of the cell membrane is presented in Fig. 2. There is a clear-cut dependence of the number of adherent cells on the radiation wavelength. Three spectral regions (600-625 nm, 645-700 nm, and 720-860 nm with the maxima around 620, 680, 750, and 825 nm) were identified where the adhesive properties of the cell membrane were enhanced. Under the conditions of our experiment the effect was most pronounced at wavelengths 680 and 825 nm (90-95% of adherent cells). At wavelengths 620 and 750 nm 80% of cells were adherent. Remember that in nonirradiated suspensions under the same experimental conditions 42% of cells were adherent, i.e., the effect of irradiation was 200-225%.

In the effective spectra of monochromatic visible and near-IR irradiation for the rate of DNA and RNA synthesis in HeLa cells in both the exponential and the stationary growth phase the maxima were found near 620, 680, 760, and 820 nm [7,8], i.e., there is a fundamental correlation between the wavelengths at which the maximums are found in all 5 effective spectra in Fig. 2 and in the spectra for DNA and RNA synthesis in cells in the exponential and stationary growth phase [7,8].

The mechanism of transduction and amplification of the photosignal, which links the light absorption by the primary photoacceptors that are components of the respiratory chain with the terminal macroeffect of irradiation - enhancement of proliferation - was proposed elsewhere [5]. This scheme involves cascades of reactions, including reactions in the cell membranes and acceleration of nucleic acid synthesis. Therefore, the fact that the effective spectra are similar at different stages of transmission and amplification of the photosignal is not surprising, and, on the contrary, confirms the validity of the proposed scheme.

As to the optimal wavelength for low-power laser therapy, the findings of the present study as well as of other studies [7,8], suggest that the regions from 820 to 830 nm or from 750 to 760 nm may be the optimal wavelengths. As is well known, light at a wavelength within these spectral regions penetrates tissues more deeply than the light at a wavelength of 680 and 620 nm [9], at which the maxima were also found in the effective spectra (Fig. 2).

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