Evaluation of the Safety of Laser-Induced Fluorescence on the Model of Human Diploid Cell Culture

O. E. Fatyukhina, T. D. Kolokoltsova*, and G. P. Troshkova

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 4, pp. 203-206, October, 2007
Original article submitted June 28, 2007

The possibility of using L-68 diploid cell culture as a model for evaluation of the safety of laser-induced fluorescence stimulated by Kr-F eximer laser (λ=248 nm) is proven. Laser irradiation of human diploid fibroblasts in a dose of 300 mJ/cm^2 and higher led to changes in cell morphology, decreased their proliferative activity and viability, and induced accumulation of lipid peroxidation products (carbonyl compounds) in the nutrient medium. Irradiation doses below 100 pulses did not modify cultural and morphological characteristics of cells.

Key Words: *cell culture; carbonyl compounds; ultraviolet irradiation; laser-induced fluorescence*

The majority of laser methods of diagnostics used in medicine are based on the fluorescence phenomenon. The method of laser-induced fluorescence (LIF) is now effectively used for the diagnosis of diseases of different etiology, evaluation of the state of visceral organ, transplanted organs, and allografts. This method effectively detects atherosclerosis of blood vessels [6] and tumors in the esophagus, uterus, lungs, airways, urinary bladder, and oral cavity [8]. Diagnostic laser methods gradually replace many invasive methods, including biopsy, expensive chromatography, and high voltage dielectrophoresis sometimes giving inadequate results.

UV radiation with a wavelength >300 nm is most often used for diagnostics. Short-wave radiation, *e.g.* λ=248 nm, provides more intense stimulation of fluorescence and the fluorescence spectra specific of each tissue type. However, exposure to UV radiation is not safe and can induce various damages at the organ, tissue, and cell levels [2].

Vector State Research Center of Virology and Biotechnology, Koltsovo, Novosibirsk region; L. A. Tarasevich Institute of Standardization and Control of Biomedical Preparations, Moscow. ***Address for correspondence:*** troshkova@vector.nsc.ru. G. P. Troshkova

Hence, the use of LIF with different stimulating radiation wavelengths for evaluation of the state of organs and tissues necessitates more profound studies of the mechanisms of interactions between UV radiation and tissues and evaluation of the safety of this method.

We evaluated safety of LIF stimulated by Kr-F eximer laser (λ=248 nm) on the model of L-68 human diploid cell culture.

MATERIALS AND METHODS

Human diploid fibroblasts L-68 were obtained from Cell Culture Museum of Vector Institute. The cells were cultured in Eagle's MEM with 5% fetal calf serum (Gibco). The cells were reinoculated after 3-4 days. A 1-2-day cell monolayer was irradiated.

Discharge Kr-F eximer laser (λ=248 nm; pulse energy 5-10 mJ/cm^2) served as the source of radiation. The duration of irradiation was 7 nsec, pulse power reaching 10^6 W. L-68 cell culture was irradiated in the following doses: 0 (control), 50, 100, 300, 500, and 700 mJ/cm^2.

The morphology of cell culture before and after irradiation in different doses was studied under an Axiovert 40 inverted-stage microscope (Zeiss).

0007-4888/07/1444-0614

After irradiation, the cells were incubated at 37°C for 5-12 h. After microscopy of irradiated cell culture and evaluation of the number of living and dead cells, the culture was reinoculated into new flasks and the nutrient medium was analyzed for the presence of carbonyl compounds (CC).

Cell viability was evaluated by trypan blue staining (0.4% solution).

The level of CC in culture fluid was evaluated by spectrophotometrically — by optical density of the nutrient medium after reaction with 2,4-dinitrophenylhydrazine at λ=505 nm as described previously [7].

RESULTS

An important aspect for studies of laser effects on cell cultures is to obtain express information on cell status, from which changes in cell metabolism at the earliest stages can be detected, before the appearance of morphological changes, cell growth delay, and decrease in cell count.

Exposure of cells to adverse factors primarily impairs lipid status in cell membranes and activates lipid peroxidation (LPO) processes [3]; LPO acceleration and release of its products (CC) is a universal response of cells to unfavorable environmental factors. CC are usually stable and can accumulate in the cell—nutrient medium system. The relationship between cell culture status and CC level in the nutrient medium was experimentally proven [7].

Changes in viability of human diploid fibroblasts after laser exposure in different doses and CC levels in the medium are summed up in Table 1.

Irradiation in doses of 300, 500, and 700 mJ/cm^2 reduced cell viability by 24, 41, and 33%, respectively. The content of CC in the medium increased by 1.8 times in comparison with the control in response to the dose of 300 cpm. Increasing the dose of laser exposure to 500-700 mJ/cm^2 led to a 2.5-fold increase in CC content. These data attest to impaired status of cell membrane lipids and LPO activation. Similar results were obtained previously [5]: high-dose UV exposure of lymphocytes led to a 1.5-2 times increase in the products of LPO reaction. The exposure dose of 100 mJ/cm^2 led to just a 12% decrease in the count of viable cells in comparison with the control. Laser exposure in a dose of 50 mJ/cm^2 did not lead to appreciable changes in the viability of irradiated cells and CC level in culture medium, which virtually did not differ from the control.

TABLE 1. Human Diploid Fibroblast Viability and CC Level in the Medium Depend on Exposure Dose (*M*±*SM*)

Laser exposure dose, mJ/cm^2	CC level, opt. dens. units, λ=505 nm (*n*=10)	Viable cells, % (*n*=10)
Control (0)	0.1221±0.0001	92±2
50	0.1251±0.0011	94±2
100	0.1289±0.0003	81±5
300	0.2313±0.0016	70±6
500	0.3343±0.0003	54±7
700	0.3643±0.0014	62±8

Hence, fibroblast culture reacts to exposure to Kr-F eximer laser. Irradiation of cell monolayer in a dose of 300 mJ/cm^2 and higher induces LPO, the concentration of CC in membranes increases with increasing the irradiation dose, which fact indicates

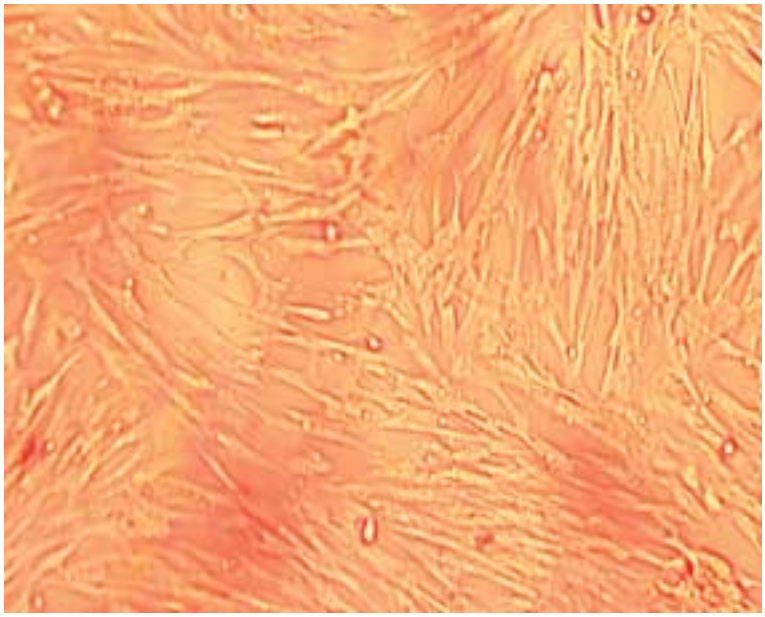

Fig. 1. Diploid fibroblast culture: unfixed unstained preparation (objective ×40).

Fig. 2. Diploid fibroblast culture after exposure to Kr-F laser in a dose of 500 mJ/cm^2 (unfixed unstained preparation; objective ×40).

the development of dose-dependent photoinduced peroxidation of unsaturated fatty acids. UV irradiation in doses below 100 mJ/cm^2 does not modify cell metabolism in L-68 human diploid cell culture.

In living systems, the physicochemical manifestations of laser exposure are paralleled by its effects on the function of living cells, which is determined by the homeostasis degree. At higher doses the cells can fail to control complete recovery of the systems, which can lead to irreversible processes and even cell death. The next stage of our work was to study the monolayer formation, morphology, and proliferation indexes of exposed human fibroblasts in order to evaluate the recovery processes.

The results indicated that the control culture was presented by typical fibroblast-like cells forming a monolayer characteristic of this culture. Round mitotic cells were detected (Fig. 1).

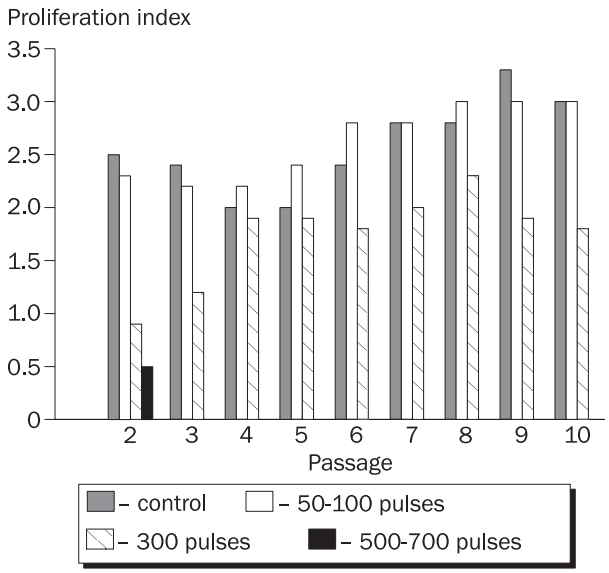

Fig. 3. Human fibroblast proliferation indexes at different laser irradiation doses.

After exposure to Kr-F laser in a dose of 300 mJ/cm^2, the culture changed the growth pattern and morphology in comparison with the control. Round compressed cells appeared in irradiated culture of diploid fibroblasts, the number of flat cells with morphology typical of this culture visually decreased in comparison with the control.

Irradiation in a dose of 500 mJ/cm^2 increased the number of round compressed cells, a negligible percentage of cells with pronounced growth were seen (Fig. 2).

Increasing the dose to 700 mJ/cm^2 reduced cell adhesion to the surface of the culture flask. Normally fibroblast-like cells acquired round shape, increased in size, and did not adhere to the flask surface.

Hence, the number of irreversible changes, after which the cells did not recover, increased with increasing the dose of laser irradiation above 300 mJ/cm^2.

Diploid fibroblasts exposed to a dose <100 mJ/cm^2 morphologically did not differ from the control.

Cell proliferation was studied in L-68 diploid cells after culturing by the standard method [1]. The diagram of proliferation indexes of L-68 diplod cell culture under the effects of different laser irradiation doses is presented in Fig. 3.

The proliferation time increased 1.6 times after cell exposure to a dose of 500-700 mJ/cm^2, the proliferation index irreversibly decreasing to the zero by passage 3. So few passages and low proliferation indexes in cells exposed to a dose of 500-700 mJ/cm^2 are explained by the unfavorable effects of high UV doses on cell culture. It is noteworthy that normally the number of passages for L-68 culture not exposed to UV radiation is about

50 (without changes in karyological characteristics).

Similarly as in response to exposure in doses of 500-700 mJ/cm^2, proliferation time increased 1.6 times in cultures exposed to 300 mJ/cm^2 and the cell proliferation indexes decreased. However, despite a trend to recovery of the culture by passage 8 (on day 24), proliferative activity of human fibroblasts did not reach the control level even by passage 10. Our results are comparable with the data of studies of diploid yeast cell recovery after UV exposure: the recovery rate was inhibited with increasing the irradiation dose, and the volume of recovery was reduced; in other words, the percentage of irreversibly damaged cells increased [5].

We found that the time of diploid fibroblast proliferation (normally 3-4 days) and fibroblast proliferation index (normally 2-3) were retained only in cells exposed to a dose no higher than 100 mJ/cm^2.

Hence, evaluation of the safety of LIF diagnostic method on the model of L-68 cell culture showed that exposure to a Kr-F eximer laser (λ=248 nm) in doses of 300 mJ/cm^2 and higher had an unfavorable effect on cell metabolism, impaired the cell membrane lipid status, activated LPO, modulated typical morphology of human fibroblasts, and reduced their proliferative activity. All these unfavorable effects of laser exposure are dose-dependent.

REFERENCES

1. R. Adams, *Cell Culture Methods for Biochemists* [in Russian], Moscow (1983).
2. P. M. Larionov, A. N. Malov, N. A. Maslov, and A. M. Orishich, *Optika Atmosfer. Okean.*, No. 2, 305-308 (2000).
3. V. P. Skulachev, *Sorosovsk. Obrazovat. Zh.*, No. 3, 4-10 (1996).
4. M. D. Tkhabisimova, L. N. Komarova, and V. G. Petin, *Tsitologiya*, **44**, No. 6, 555-559 (2002).
5. V. E. Kholmogorov, V. A. Krylenkov, and M. A. Osmanov, *Biological Effect of Ultraviolet Radiation* [in Russian], Moscow (1975), pp. 164-177.
6. J. J. Baraga, R. P. Rava, and P. Taroni, *Lasers Surg. Med.*, **10**, No. 3, 245-261 (1990).
7. O. E. Fatyukhina, G. P. Troshkova, T. D. Kolokoltsova, *et al.*, *Biotechnology and Medicine*, Nova Science Publishers (2004).
8. C. Y. Wang, H. K. Chiang, C. T. Chen, *et al.*, *Oral Oncol.*, **35**, No. 2, 144-150 (1999).