REVISTA ESPAÑOLA DE FISIOLOGIA, 51 (1), 43-48, 1995

He-Ne Laser has no Effect on Cell Cycle Phases of Human Colon Adenocarcinoma Cells

J. A. Morales, M. J. Ruiz-Gómez, L. Gil-Carmona,
A. Souvirón and M. Martínez-Morillo

Departamento de Radiología y Medicina Física, Facultad de Medicina,
Universidad de Málaga, 29071 Málaga (Spain)

(Received on October 18, 1994)

J. A. MORALES, M. J. RUIZ-GÓMEZ, L. GIL-CARMONA, A. SOUVIRÓN and M. MARTÍNEZ-MORILLO. *He-Ne Laser has no Effect on Cell Cycle Phases of Human Colon Adenocarcinoma Cells.* Rev. esp. Fisiol. (J. Physiol. Biohem.), 51 (1), 43-48, 1995.

The aim of this study is to investigate the effect of He-Ne continuous laser (12.6 mW, 632.8 nm), at low energy densities, on cell cycle synchronization of monolayer growing human colon adenocarcinoma cell line. The doubling time of cell culture was used as optimum time to verify laser effect. The monolayer cultures were exposed to single doses of different energy densities (0.042 J cm^{-2} to 1.68 J cm^{-2}). The nuclear DNA content has been studied by flow cytometry to obtain the cell percentage in each cell cycle phase. Results show no effect of He-Ne laser irradiation on cell cycle short time synchronization under the previously mentioned conditions and cell type. Higher energy densities and multiple irradiations should be investigated.

Key words: Laser irradiation, Monolayer cell line, DNA.

Low energy He-Ne laser irradiation has been used with different effects that are not well defined. Photodynamic therapy, bioregulation, acupuncture and wound healing are the most common uses and applications of low power laser. Mechanisms of these effects are still under investigation (6).

It has been shown in biological studies that low power laser irradiation can stimulate DNA and RNA synthesis and increase respiratory activity in mitochondria (1, 7). He-Ne laser irradiation can activate rRNA synthesis of human peripheral lymphocytes (9). In addition, dose-response studies have shown that repeated laser irradiation once a day (11.9 J cm^{-2} to 142 J cm^{-2}) can inhibit cell growth, while a single irradiation with an energy density of 142 J cm^{-2} can increase the number of cells in mitosis (3).

On the other hand, doses of 1.2 J cm^{-2} have been shown to induce cell growth

Correspondence to A. Souvirón (Tel.: 95 - 213 15 78; Fax: 95 - 213 16 30).

acceleration. The greatest effect was obtained four days after treatment. When cells were submitted to repeated irradiations every four days, cellular divisions were increased (11).

Results published up to now suggest that laser irradiation could induce modifications on cell cycle phases. In this work we studied He-Ne laser effects on human colon adenocarcinoma cell cycle after exposure to different energy densities. DNA content was measured by flow cytometry to distinguish alterations in cell percentage in each phase (G_1, S, G_2+M). Flow cytometry is an objectively measureable end point of laser irradiation.

Materials and Methods

Cell culture.– Human colon adenocarcinoma cells (cell line provided from Biochemistry Deptment) were grown in RPMI-1640 medium (with L-glutamine calcium and magnesium free) and supplemented with hepes buffer 1 M (15 ml L^{-1}), sodium bicarbonate 7.5 % (28 ml L^{-1}), 10 % heat inactivated calf serum and 1 % antibiotic-antimycotic solution 100 X (PSF, Gibco); at 37 °C in a 5 % CO_2/air atmosphere. These cells grow in monolayer and are subcultivated using trypsin (0.05 %) and EDTA (0.02 %) in Dulbecco's phosphate-buffered saline (PBS) (2, 5).

Laser irradiation.– Irradiations were performed with low-power continuous He-Ne laser (632.8 nm, 12.6 mW, J&JE). The beam was defocused to irradiate 9 cm^2 (fig. 1). During irradiation the media did not contain red pH indicator dye. Energy density varied from 0.042 J cm^{-2} to 1.68 J cm^{-2}. Control cells were sham-irradiated.

Culture plates used were 6-well type. In each well, 2 ml of supplemented RPMI-1640 medium containing 500,000 cells was inoculated. They were incubated for 24 h at 37 °C and then exposed to laser irradiation in the absence of light after opening the plate cover. Three black opaque templates were set under, surrounding and the last one, with a centered hole, on the plate. They were used to protect the adjacent wells from scattered laser irradiation (6). After irradiation, culture medium was removed and 2 ml of fresh supplemented medium at 37 °C was inoculated. Cells were incubated for 17 h 30 min (doubling time of cell culture, fig. 2) and then harvested and processed to measure DNA content.

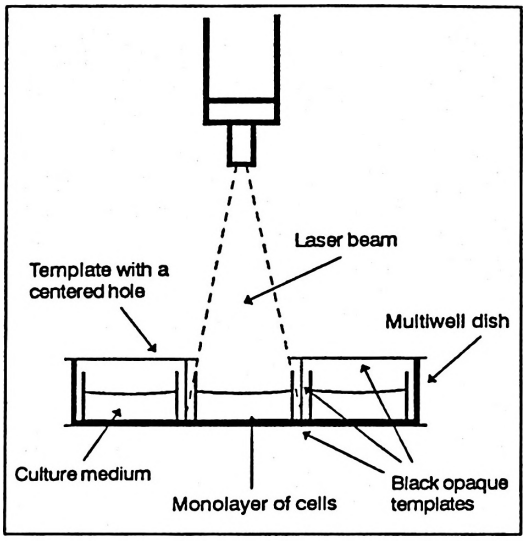

Fig. 1. *Irradiation procedure.*
Laser beam was defocused to irradiate 9 cm^2. Cells were exposed to laser irradiation in the absence of light after opening the plate cover. Three black opaque templates were set under, surrounding and the last one, with a centered hole, on the plate. They were used to protect the adjacent wells from scattered laser irradiation. The media did not contain red pH indicator dye.

DNA content.– After harvesting medium and monolayer, cells were washed

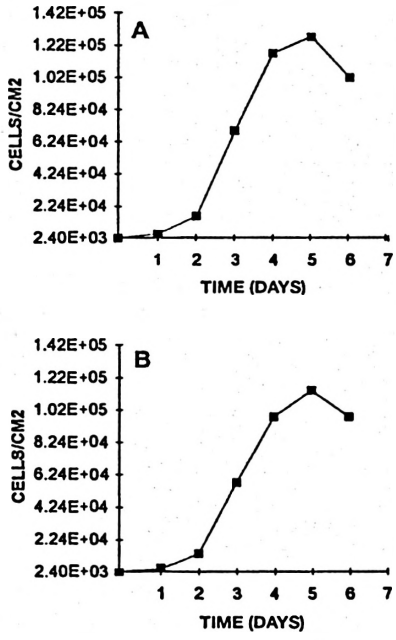

Fig. 2. *Growth curves of adenocarcinoma cells in monolayer.*
Two growth curves were assayed with different concentrations of heat inactivated calf serum (HICS) to determine the optimum doubling time: A) 10 % HICS, doubling time: 17 h 30 min, and B) 5 % HICS, doubling time: 19 h 35 min.

twice in PBS and centrifuged at 1500 rpm for 5 min. Pellets were fixed in 70 % ethanol/PBS at 4 °C overnight.

Cell suspensions were washed in PBS, centrifuged and resuspended in 0.9 ml at 37 °C. After cooling, 0.1 ml of propidium iodide (125 µg ml^{-1}) was added and then incubated in the dark at 4 °C for 30 min (4, 8, 10).

DNA content was quantified on a flow cytometer (FACScan, Becton Dickinson). Histograms were prepared by using a minimun of 15,000 cells and cell aggregates were gated out before the two peaks (G_1 and G_2+M) were drawn.

Statistical analyses of the data were done with the Student's *t* test.

Results and Discussion

The development of flow cytometry has made possible to distinguish, by means of a fluorescent stain (propidium iodide), cell populations with different DNA content and therefore, the cell cycle phases.

The average coefficient of variation of G_0/G_1 peaks was 3.28 ± 0.7 % and the $(G_2+M)/G_1$ ratio was 1.95.

The data show no alteration on percentages of cells in each phase after being irradiated with different energy densities from 0.042 to 1.68 J cm^{-2} (table I).

The results are in disagreement with the studies reported by YEW *et al.* (12) who found a decrease in leucine uptake and a temporary decrease in the number of mitoses after irradiation with He-Ne laser (11, 22, 33 mJ cm^{-2}) on rat astrocytes. GROSS *et al.* (3) stated that the number of cells in mitosis increases after single irradiation with 142 J cm^{-2} and SOUDRY *et al.* (11) found an increase in cellular divisions after repeated doses with 1.2 J cm^{-2}. GROSS *et al.* (3) and YEW *et al.* (12) found a growth inhibition whereas SOUDRY *et al.* (11) are in disagreement with this finding, observing a growth acceleration. These findings indicate that the effects of He-Ne laser are different in different cell lines and depend on laser treatment.

No effect, however, was found on cell cycle phases distribution. This may be due to the absence of response to He-Ne laser on this human colon adenocarcinoma cell line. In this way, JUI-CHANG and MING-CHIEN (6) observed a biostimulatory effect on C6 glioma cells (2.7 to 81 mJ^{-2}) but they found no effect on RBA-1 cells at the same doses. Another explanation could be the low energy densities applied. Biostimulation depends on energy density, irradiation procedures and cell type, the last probably being the most important factor.

Table I. *Cellular percentages in each cell cycle phase.*
The monolayer cultures were irradiated with low-power continuous He-Ne laser (42 to 1680 mJ cm^{-2}). Control cells were sham-irradiated. After irradiation, culture medium was changed, cells were incubated for 22 h and then harvested and processed to measure DNA content on a flow cytometer to distinguish alterations in cell cycle phases (Mean ± SD of four replicates). ED = Energy density (mJ cm^{-2}); I = Irradiated groups; CTL = Control groups.

ED	G_1 Phase		S Phase		G_2 + M Phase	
	I	CTL	I	CTL	I	CTL
42	56.13 ± 1.75	53.03 ± 1.66	27.83 ± 3.37	31.20 ± 4.18	16.07 ± 2.04	15.80 ± 3.05
84	56.60 ± 1.48	58.73 ± 1.52	25.07 ± 1.58	23.00 ± 2.42	18.33 ± 0.69	18.27 ± 0.90
168	56.97 ± 3.51	54.27 ± 2.45	22.50 ± 3.54	24.73 ± 4.69	20.57 ± 0.17	21.03 ± 2.25
336	48.07 ± 3.12	49.67 ± 6.62	33.63 ± 4.46	33.67 ± 7.01	18.33 ± 1.40	16.67 ± 1.19
840	49.50 ± 2.05	48.80 ± 4.49	31.33 ± 2.11	31.63 ± 10.16	19.13 ± 0.49	19.53 ± 5.72
1680	48.43 ± 2.66	48.43 ± 2.98	32.60 ± 5.24	34.93 ± 4.08	18.93 ± 2.62	16.67 ± 1.24

An objectively measurable technique (flow cytometry) that allows the quantification of laser effects has been used unlike other papers.

We conclude that He-Ne laser did not cause, at low energy densities, alterations in the percentages of cells in each cell cycle phase of this hurnan colon adenocarcinoma cell line. Furthermore, exposed He-Ne laser irradiation did not synchronize the cell cycle phases.

Acknowledgements

The authors would like to acknowledge Prof. S. Perán at the "Departamento de Bioquímica de la Facultad Medicine de la Universidad de Málaga" (Spain), for his generosity in providing the colon adenocarcinoma cells. We thank Dr. F. Alvarez for his kindness in reading the manuscript.

J. A. MORALES, M. J. RUIZ-GÓMEZ, L. GIL-CARMONA, A. SOUVIRÓN y M. MARTÍNEZ-MORILLO. *El láser He-Ne no afecta a las fases del ciclo celular en adenocarcinoma de colon humano.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (1), 43-48, 1995.

Se estudia el efecto del láser He-Ne de emisión continua (12,6 mW, 632,8 nm), a bajas densidades de energía sobre la sincronización del ciclo celular de células de adenocarcinoma de colon humano cultivadas en monocapa. El tiempo de duplicación celular del cultivo se usa como el tiempo óptimo para verificar los efectos del láser. Los cultivos en monocapa se exponen a dosis únicas de diferentes densidades de energía (de 0,042 J cm^{-2} a 1,68 J cm^{-2}). El contenido nuclear de ADN se determina por citometría de flujo para obtener el porcentaje celular en cada fase del ciclo. Los resultados no muestran efecto de la irradiación láser He-Ne sobre la sincronización a corto plazo del ciclo celular, bajo las condiciones y tipo celular ensayados.

Palabras clave: Irradiación por láser, Línea celular en monocapa, DNA.

References

1. Fedoseyeva, G. E., Smolyaninova, N. K., Karu, T. *et al.* (1988): *Lasers Life Sci.*, 2, 197-205.
2. Griffiths, B. (1986): In "Animal cell culture: a practical approach" (Freshney, R. I., ed.). IRL Press. Oxford. pp. 33-69.
3. Gross, A. J. and Jelkmann, W. (1990): *Lasers Surg. Med.*, 10, 40-44.
4. Hata, Y., Ishizu, H., Ohmori, K. *et al.* (1991): *Cancer*, 68, 2566-2570.
5. Hein, R., Landthaler, M., Haina, D. and Krieg, T. (1992): *Lasers Med Sci.*, 7, 79-83.
6. Jui-Chang Tsai and Ming-Chien Kao (1991): *J. Clin. Laser Med. Surg.*, 9, 35-41.
7. Karu, T. (1989): *Health Phys.*, 56, 691-704.

8. Kimura, H., Yonemura, Y. and Epstein, A. L. (1991): *Cancer,* 68, 2175-2180.
9. Manteifel, V. M., Andreichuk, T. N., Karu, T. I. *et al.* (1990): *Mol. Biol.* (Mosk), 24, 1067-1075.
10. Orrnerod, M. G. (1990): In "Flow cytometry: a practical approach". IRL Press, Oxford. pp. 69-86.
11. Soudry, M., Franquin, J. C., Pourreau-Schreider, N. and Martin, P. M. (1988): *J. Biol. Buccale,* 16, 129-135.
12. Yew, D. T., Zheng, D. R., Au, C. and Li, W. W. Y. (1990): *Scanning Microsc.,* 4, 151-159.