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

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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.
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 (G1, S, G2+M).

Flow cytometry is an objectively measurable 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 % CO₂/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&J). The beam was defocused to irradiate 9 cm² (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.

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

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Fig. 1. Irradiation procedure.
Laser beam was defocused to irradiate 9 cm². 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 medium did not contain red pH indicator dye.
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 G2/G1 peaks was 3.28 ± 0.7 % and the (G2+M)/G1 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 SOURDY 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 SOURDY 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 mJ²) 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.

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 minimum of 15,000 cells and cell aggregates were gated out before the two peaks (G1 and G2+M) were drawn.

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

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Fig. 2. Growth curves of adenocarcinoma cells in monolayer.
Two growth curves were assayed with different concentrations of heat inactivated cell 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.
Table I. Cellular percentages in each cell cycle phase.

<table>
<thead>
<tr>
<th>ED</th>
<th>G1 Phase</th>
<th>S Phase</th>
<th>G2 + M Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (mean ± SD)</td>
<td>I (mean ± SD)</td>
<td>I (mean ± SD)</td>
</tr>
<tr>
<td>42</td>
<td>56.13 ± 1.75</td>
<td>27.83 ± 3.37</td>
<td>16.07 ± 2.04</td>
</tr>
<tr>
<td>84</td>
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<tr>
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<td>48.43 ± 2.66</td>
<td>32.80 ± 5.24</td>
<td>18.93 ± 2.02</td>
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</tbody>
</table>

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 human colon adenocarcinoma cell line. Furthermore, exposed He-Ne laser irradiation did not synchronize the cell cycle phases.

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