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ORIGINAL ARTICLES

EFFECT OF 630-NM PULSED LASER IRRADIATION ON THE PROLIFERATION OF HeLa CELLS IN PHOTOFRIN®-MEDIATED PHOTODYNAMIC THERAPY

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Background and Aims: Red laser light of wavelength 630 nm is usually used for Photofrin®-mediated photodynamic therapy (PDT). The 630-nm light employed in PDT corresponds to the region of the wavelength used in low-level laser therapy (LLLT) may influence on the photodynamic effect required for killing cancer cells. The aim of this in vitro study was to investigate the changes in cell viability and degree of cell proliferation after Photofrin®-mediated PDT using 630-nm pulsed laser irradiation (10 Hz repetition rate and 7-9 ns pulse width), which was clinically found to induce no remarkable cell injury.
Materials and Methods: A study has been conducted in which HeLa cells are incubated with Photofrin® for 15 min (10 μg/ml). Irradiation was carried out at an average fluence rate of 50 mW/cm^2 with light doses of 1, 3, and 5 J/cm^2. The cytotoxic effects on the cells are evaluated by the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) assay.
Results: The results showed that the laser irradiated cells exhibited a greater clonogenic activity than normal and PDT treated cells for a short period after the laser irradiation.
Conclusion: If the level of 630-nm pulsed laser irradiation employed in a PDT is comparatively lowered, it would have a biostimulatory effect like that of in LLLT.

Key Words: PDT, LLLT, HeLa cells, Cell proliferation, 630-nm pulsed laser

Introduction

Photodynamic therapy (PDT) employs the interaction of a photosensitizer with light of the appropriate wavelength in the presence of molecular oxygen and is used to treat malignant tumors. [1)]

Photofrin® is a type of first-generation clinical PDT that has been used to treat various cancers. [2)] Red laser light of wavelength 630 nm is usually used for Photofrin®-mediated PDT. [3)] This wavelength is used because (1) the Photofrin® Q-band exists at this wavelength and (2) a comparatively higher penetration of light may be achieved. Moreover, high-intensity 630-nm pulsed laser irradiation has also been used in Japan, and the therapeutic effects observed are superior to those obtained with irradiation at other wavelengths. [4)]

It is well known that wavelength of approximately 630 nm represents the biostimulatory region of visible light and promotes cell proliferation, DNA synthesis, and cell adhesion. [5,6)] These phenomena are thought to be induced by the absorption of light by cytochrome c oxidase present in the mitochondria, which has an absorption band ranging from 600 to 1100 nm. [7)] Cytochrome c oxidase is considered a pri-

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Manuscript received: March 16th, 2011
Accepted for publication: May 18th, 2011

mary photo-acceptor in low-level laser therapy (LLLT). [8] Thus, the 630-nm light used in PDT corresponds to the wavelength of light used in LLLT may influence on the photodynamic effect required for killing cancer cells.

The aim of this study was to investigate the changes in cell viability and degree of cell proliferation after PDT using 630-nm pulsed laser irradiation, which was clinically found to induce no remarkable cell injury.

Materials and Methods

Cell culture

HeLa cells were cultivated at 37°C in Ham's F-10 medium (Cosmo Bio Co., Japan) supplemented with antibiotics and 10% fetal bovine serum. Cells in the log phase of growth were used. The cells were seeded into a 96-well flat-bottomed culture plate at a density of 4.2×10^4 cells/well and were incubated overnight at 37°C.

Photodynamic therapy

The medium in each well was then replaced with 10 μg/mL Photofrin®(Pfizer Inc., Japan)-containing Dulbecco's PBS (-), the cells were incubated for 15 min, and they were then rinsed twice with PBS (-). The cells were then irradiated in the buffer by using an Nd:YAG-pumped optical parametric oscillator (OPO) that has a pulse repetition rate of 10 Hz and a pulse width of 7–9 ns (model MOPO-710, Spectra Physics, USA). The wavelength of the laser was 630 nm. Irradiation was carried out at an average fluence rate of 50 mW/cm^2 (i.e., ~5 mJ/cm^2 OPO pulse) with light doses of 1, 3, and 5 J/cm^2.

Experimental groups

Cells treated with Photofrin® (10 μg/mL) but not irradiated with 630-nm laser light were used as the control (Control). Cells not treated with Photofrin® but irradiated with the laser were used as the laser-irradiated control (LC). After the irradiation, the PBS (-) in the wells was replaced with the culture medium, and the cells were incubated for some time before each assessment.

Cell viability assay

Cell viability was assessed using the XTT (2, 3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) assay, which yields similar results to those obtained from clonogenic assays. [9] Ten microliters of XTT solution (Cell Counting Kit-8; Dojindo, Japan) was added to the wells at each assessment time (0, 6, 12, and 24 h after PDT), and the cells were incubated at 37°C for 2 h. The absorbance was read at 470 nm using a microplate reader (iMark Microplate Reader; Bio-Rad Lab., USA). For each assessment, absorbance data were normalized with those obtained for Control or LC. Data were represented as mean ±SD. Statistical analysis was performed using Student's t test. Differences were considered to be statistically significant at $P < 0.05$.

Results and Discussion

Figure 1 shows the effect of each light dose (1, 3, and 5 J/cm^2) on cell viability measured using XTT assay as a function of post-irradiation time. The cells were exposed to 630-nm radiation at a fluence rate of 50 mW/cm^2. The activities were calibrated using (1) cells treated with Photofrin® but not irradiated (Control) and (2) cells irradiated with the laser but not treated with Photofrin® (LC).

The cell viability was found to decrease with the increasing light dose. In addition, there was a gradually decrease in the cell viability during the post-irradiation time. These results agree with those of a previous report which stated that a low level pulsed laser excitation during PDT would induce cell death due to cell cycle-dependent apoptosis. [10]

The cell viability values normalized using Control were higher than those normalized using LC for any light dose, and this difference was prominent during the short period of post-irradiation time. Significant differences in cell viability were observed at 0 h (1, 3, 5 J/cm^2) and 6 h (5 J/cm^2) after PDT. These findings suggest that the viability of the cells irradiated with 630-nm pulsed laser light is greater than that of the non-irradiated cells.

Several investigators have shown that red and near-infrared radiation can activate certain cellular responses. [11] Recently, a part of the mechanism responsible for this activation has been revealed. Karu and colleagues examined whether HeLa cells irradiated with monochromatic light of wavelength 580–860 nm showed an increased rate of DNA synthesis and cell adhesion. [12] They found that irradiation with light of wavelength 620 nm could promote DNA synthesis and cell adhesion. The wavelength described is almost equal to the excitation wavelength of laser light used in Photofrin®-mediated PDT in the current study.

The results presented here suggest that the proliferation of HeLa cells irradiated with 630-nm pulsed laser light increases in the early post-irradiation time over that of non-irradiated cells; i.e., the LLLT effect is

observed when the irradiation level is sufficiently low, even if high-intensity pulsed laser irradiation is used like in the case of clinical PDT. Since clinical PDT employs light doses that are 10–100 times those employed in this study, we could ignore the LLLT effect observed here. [4,13] In PDT, 630-nm laser irradiation of the body parts where light dose becomes remarkably low, e.g., deep within a tissue, may produce the LLLT effect and not the early cytotoxic effect. [14,15]

Acknowledgement

This work was supported by Grant-in-Aid for Research Activity Start-up (No. 22800059), Japan. This work was also partially supported by SMU-FHMC Grant 10-01 (No. 025), Japan. We are grateful to Dr. Tsuyoshi Nishisaka for his technical support.

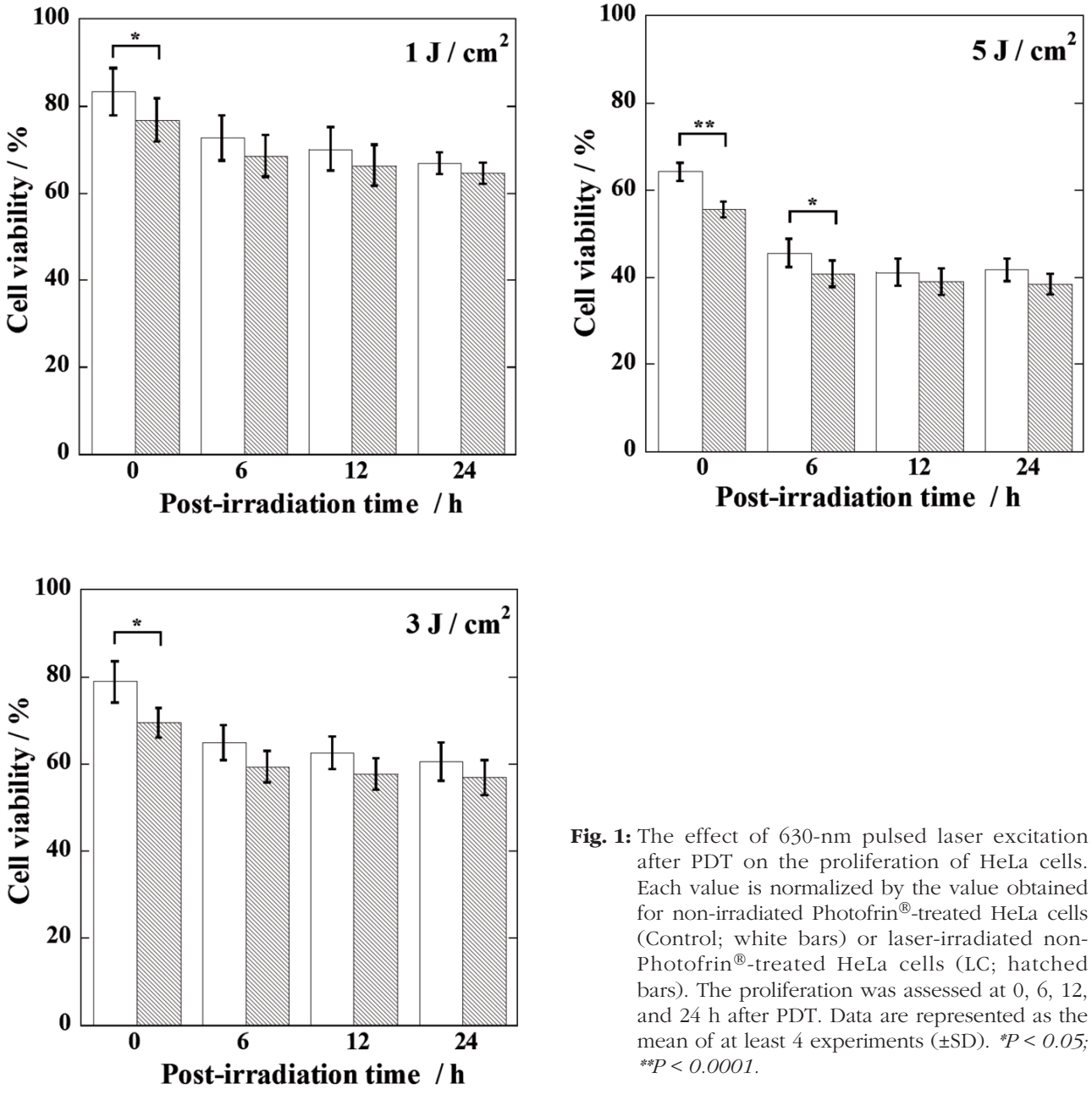

Fig. 1: The effect of 630-nm pulsed laser excitation after PDT on the proliferation of HeLa cells. Each value is normalized by the value obtained for non-irradiated Photofrin®-treated HeLa cells (Control; white bars) or laser-irradiated non-Photofrin®-treated HeLa cells (LC; hatched bars). The proliferation was assessed at 0, 6, 12, and 24 h after PDT. Data are represented as the mean of at least 4 experiments (±SD). **P < 0.05; **P < 0.0001.*

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