Journal of Photochemistry & Photobiology, B: Biology 160 (2016) 271–277

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Photodynamic therapy of HeLa cell cultures by using LED or laser sources

María E. Etcheverry [a,b], Miguel A. Pasquale [b], Mario Garavaglia [a,c,*,1]

[a] Departamento de Física, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), Argentina
[b] Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA) (CCT CONICET La Plata, UNLP y CIC), Argentina
[c] Centro de Investigaciones Opticas (CIOp) (CCT CONICET La Plata y CIC), Argentina

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 6 April 2016
Accepted 11 April 2016
Available online 16 April 2016

Keywords:
Photodynamic therapy
HeLa cultures
LED vs LASER
m-THPC

ABSTRACT

The photodynamic therapy (PDT) on HeLa cell cultures was performed utilizing a 637 nm LED lamp with 1.06 W power and m-tetrahydroxyphenyl chlorin (m-THPC) as photosensitizer and compared to a laser source emitting at 654 nm with the same power. Intracellular placement of the photosensitizer and the effect of its concentration (C_P), its absorption time (T_A) and the illumination time (T_I) were evaluated. It was observed that for $C_P > 40$ μg/ml and $T_A > 24$ h, m-THPC had toxicity on cells in culture, even in the absence of illumination. For the other tested concentrations, the cells remained viable if not subjected to illumination doses. No effect on cells was observed for $C_P < 0.05$ μg/ml, $T_A = 48$ h and $T_I = 10$ min and they continued proliferating. For drug concentrations higher than 0.05 μg ml^{-1}, further deterioration is observed with increasing T_A and T_I. We evaluated the viability of the cells, before and after the treatment, and by supravital dyes, and phase contrast and fluorescence microscopies, evidence of different types of cell death was obtained. Tetrazolium dye assays after PDT during different times yielded similar results for the 637 nm LED lamp with an illuminance three times greater than that of the 654 nm laser source. Results demonstrate the feasibility of using a LED lamp as alternative to laser source. Here the main characteristic is not the light coherence but achieving a certain light fluence of the appropriate wavelength on cell cultures. We conclude that the efficacy was achieved satisfactorily and is essential for convenience, accessibility and safety.

1. Introduction

Photodynamic Therapy (PDT) centers on the photochemical interaction of three principal components: light, photosensitizer and oxygen [1]. This treatment modality uses light of an appropriate wavelength in the presence of oxygen to activate a photosensitizing drug, which then causes localized cell death or tissue necrosis. Singlet oxygen ($^1O_2$) is generally believed to be the major cytotoxic agent during PDT. In 1978, Thomas J. Dougherty conducted the first trials of PDT with Hematoporphyrin Derivative (HpD) in humans using 633 nm laser radiation. PDT has been approved by the US Food and Drug Administration for the treatment of microinvasive lung cancer, obstructing lung cancer and obstructing esophageal cancer, as well as premalignant affections such as actinic keratosis and macular degeneration associated with aging. Studies have shown efficiency in the treatment of head and neck cancer [2,3], lung cancer [4,5,6], mesothelioma [7], Barrett's esophagus [8,9], prostate [10,11,12] and brain [9,13,14,15] tumors. Unlike radiation therapy, PDT uses non-ionizing radiation, which can be administered repeatedly without generating cumulative effects because the target does not seem to be the DNA. The PDT involves a dynamic process, i.e., the distribution of light is determined by the source luminous characteristics and tissue optics, which, in turn, are influenced by the concentration of the photosensitizer and oxygen in the tumor. The distribution of oxygen is altered by the photodynamic process that consumes oxygen and, finally, the distribution of the photosensitizer can change as a result of photobleaching. All this, leads to the following difficulties in the practical application of PDT [16]: 1) the exact determination of the amount of photoactive drug in the tumor and its internal distribution; and 2) precise determination of the amount of light energy in the affected volume delivered from sources. In this respect, considerable progress has been made in understanding the basic of the processes and in developing devices to measure the relevant magnitudes to optimize the PDT treatment [17,18].

In general, any source that emits in the adsorption spectrum range of the photosensitizer, which is able to penetrate enough in tissues and with an appropriate light power can be used in PDT. However, there is in vitro evidence that flash wave (FW) xenon lamps, especially at low frequency of irradiation, has a photodynamic efficiency greater than continuous wave (CW) lights, a fact that could be explained by the greater generation of $^1O_2$ in the cells [19]. Although, the use of different types of light sources has been reported, a further quantitative

* Corresponding author at: Centro de Investigaciones Opticas (Ciop), Camino Centenario and 506 Street, Gonnet, La Plata, Argentina.
E-mail address: garavagliam@ciop.unlp.edu.ar (M. Garavaglia).
[1] Postal address: CC3, 1897 Gonnet, La Plata, Argentina.

http://dx.doi.org/10.1016/j.jphotobiol.2016.04.013
1011-1344/

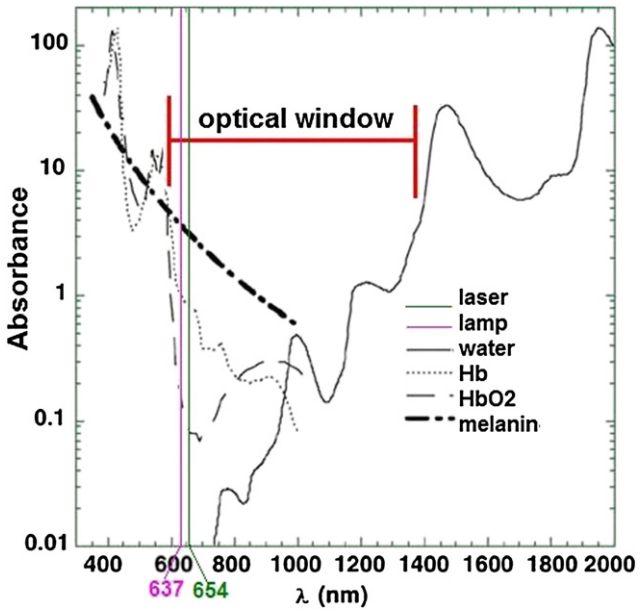

Fig. 1. Optic window and the emission wavelengths of the sources employed in the experiments. This figure was adapted from its original in reference [22] to show the spectral location of the laser and the LED emissions used in this work.

comparison of their efficiency is needed [20]. Several experiments that take into account the fluence of light and the concentration of the photosensitizer have revealed the existence of a photodynamic threshold. This is defined as the minimum number of photons to be absorbed by the photosensitizer per unit volume of tissue to produce necrosis [21].

The differences between the incident irradiance and in situ fluence rate should be considered due to the contribution of scattered light. However, in practice the dose of light on the surface is specified using the fluence (J/cm^2) regardless of scattered photons. The light fluence is calculated from the output of the light source multiplied by the exposure time. Some protocols give the light dose prescribed as a measure of in situ fluence. Besides, fluorescence measurements provide additional information on the concentration and distribution of the photosensitizer, while the diameter of the incident beam affects the luminous flow rate at all depths. In the present study, we determined the photodynamic efficacy for HeLa cells cultivated in vitro with m-tetrahydroxyphenyl chlorin (m-THPC) using a LED lamp emitting at 637 nm with a power of 1.06 W. These results were compared with those obtained with a clinically used laser.

Table 1
Features and conditions for the use of m-THPC [23].

Properties	Photosensitizer: m-THPC
Absorption maximum (nm)	652
Absorption coefficient (cm^{-1} mol^{-1} L)	22,400
Drug Dose (mg/kg)	0.1–0.15
Absorption time (h)	96
Fluence (J/cm^2)	10–20
Fluence rate (mW/cm^2)	100

2. Materials and Methods

2.1. Light Sources

Human tissue is a highly absorbing and highly scattering medium in which biological chromophores define an optic window. The wavelength range necessary to activate the photosensitizing drug is typically between 600 nm and 800 nm (Fig. 1).

Two illumination sources were utilized in the experiments performed here:

- Laser of 654 nm ± 2.4 nm and varied emitting power up to 1.1 W (Section 4). The light emitted by the laser was coupled through an optical fiber whose free end has a microlens that makes the beam diverge illuminating in a circular area.
- LED lamp (Fig. 2) of 637 nm ± 18.1 nm and emitting power of 1.06 W.

The illuminance of the light sources at different distances was measured placing the lamp in an optical bench and employing an illuminance meter. The source emission spectrum was obtained utilizing an Ocean Optics HR2000 + ES.2 spectrophotometer.

2.2. Cell Culture

HeLa cells (50–60 passages) were grown in an atmosphere of 5% CO_2, at 37 °C and 97% of humidity in RPMI medium containing 10% fetal bovine serum (FBS) and sodium bicarbonate. HeLa cell cultures were prepared by seeding 50,000–100,000 cells into polystyrene Petri dishes 3.5 cm in diameter (Geiner bio-one). All procedures for maintenance of cells and preparation of the cultures were performed under sterile conditions using a laminar flow.

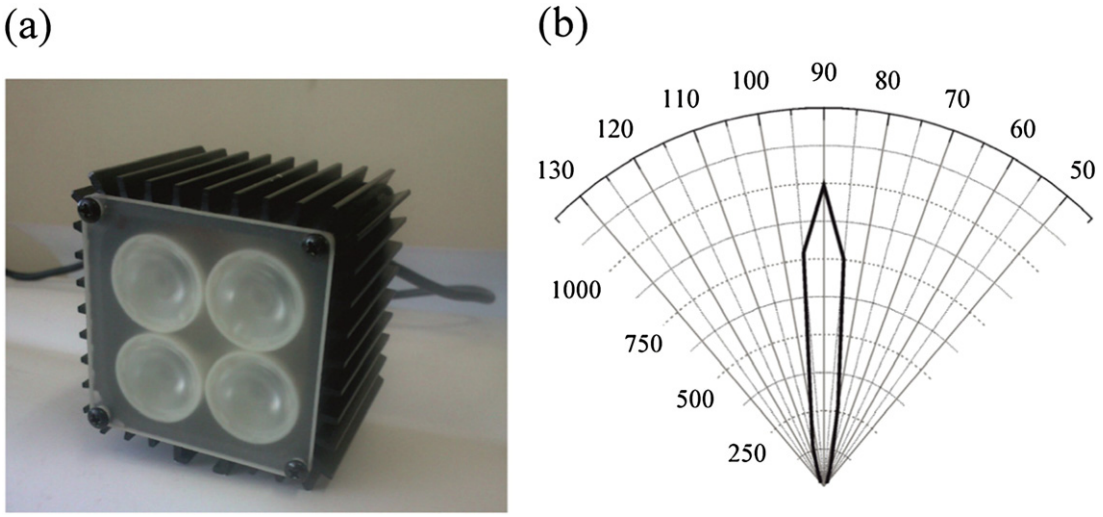

Fig. 2. LED lamp with its characteristic photometric curve.