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Synergistic effect of photodynamic therapy and cisplatin: A novel approach for cervical cancer

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ABSTRACT

Cervical cancer is a neoplasia primarily caused by Human papillomavirus (HPV) infection. Current treatment modalities involve cisplatin, a potent chemotherapeutic agent with severe adverse effects. Photodynamic therapy (PDT) is a promising modality for the treatment of cancer and infections, which has been associated with innovative therapeutic approaches, especially for the treatment of neoplasias. This study aimed to investigate the anticancer potential of PDT mediated by methylene blue (MB) or Photogem (PG) individually and combined with cisplatin *in vitro*. SiHa, C-33 A and HaCaT cells were incubated with MB, PG and/or cisplatin and received no further treatment or were irradiated with a 630 or a 660 nm LED light source at energy densities varying according to the photosensitizer (PS). The MTT assay was employed to assess cell viability. Both PS were effective in reducing cell viability with the cytotoxicity being dependent on the light dose. When compared to PDT groups, cisplatin was less effective. The cell viability of the combined therapy groups was significantly lower compared to monotherapies. The sequence of treatments (PDT + cisplatin/cisplatin + PDT) was important and had different results when varying the PS, but combination therapy resulted in an enhanced anticancer effect regardless of treatment protocol.

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1. Introduction

Cervical cancer is the third most common cancer among women worldwide with 530,232 new cases diagnosed and 275,008 deaths occurring annually, according to 2008 data [1]. HPV infection is considered the causal factor of cervical cancer with viral DNA being detected in 99.7% of cases [2]. The best way to prevent HPV infection is through vaccination, and two vaccines have been commercially available since 2006 [3]. The persistent viral infection leads to intraepithelial transformations of an insidious and progressive nature that culminates in a carcinoma if left untreated [4]. Lesions can be easily treated and completely cured if identified at an early stage [3]. If the lesions are not identified at an early stage, the treatment protocol is dependent on the tumor stage and is usually based on the association of at least two treatment modalities, such as chemotherapy/surgery or chemotherapy/radiotherapy [5,6].

Almost all protocols for cervical cancer treatment are cisplatin-based either as a single drug regimen or associated with radiation or other antineoplastics [5]. Cisplatin forms DNA adducts resulting in the inhibition of DNA replication, thus activating apoptosis. Cisplatin's mechanism of action makes it a potent chemotherapeutic agent, but the associated adverse effects, such as nephrotoxicity and ototoxicity, as well as the resistance shown by several tumors limit its clinical use; therefore, cisplatin is combined with other therapies to achieve a desirable clinical outcome [7,8].

Photodynamic therapy (PDT) is a treatment modality capable of eliminating fast-proliferating cells that involves the administration of an individually non-toxic photosensitizer (PS) and its activation by light of a specific wavelength in the presence of oxygen, thus leading to cell and tissue toxicity via oxidative damage [9,10]. PDT has been successfully employed in cancer treatment, and it has several advantages, such as few adverse effects, minimal invasiveness and double selectivity [11].

Combining PDT and cisplatin can sum the advantages of each individual treatment, improve cervical cancer treatment and reduce cisplatin toxicity [12,13]. Therefore, the aim of this study was to evaluate the effect of PDT and cisplatin individually and in combination on cervical carcinoma cells infected and non-infected with HPV16 *in vitro*.

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2. Materials and methods

2.1. Cell cultures

The cell lines used in this study were SiHa (cervical carcinoma infected with HPV16; ATCC® HTB35™), C-33 A (cervical carcinoma not infected with HPV; ATCC® HTB31™), and HaCaT (spontaneously immortalized human keratinocytes). All cell lines were grown in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM, Sigma Co., St. Louis, USA) and Ham's Nutrient Mixture F10 (Sigma Co., St. Louis, USA) supplemented with 10% fetal bovine serum (FBS; Cultlab, Campinas, Brazil), 1X antibiotic/antimycotic solution (100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B; Sigma Co., St. Louis, USA) and 0.1 mg/mL kanamycin (Sigma Co., St. Louis, USA), which is referred to as complete medium hereafter. Cells were kept in 5% CO_2 atmosphere, 95% relative humidity and a constant temperature of 37 °C.

2.2. Photosensitizers and cisplatin

Photogem (Photogem LLC. Co., Moscow, Russia; Fig. 1A) was dissolved in PBS at a concentration of 5 mg/mL and was stored at −20 °C. Methylene blue (Sigma Co., St. Louis, USA; Fig. 1B) was dissolved in PBS at a concentration of 10 mg/mL and stored at −20 °C. Both PSs were diluted to working concentrations in complete medium prior to use.

Cisplatin was obtained as a 0.5 mg/mL solution (Tecnoplatin, Zodiac Produtos Farmaceuticos S/A, Brazil) and stored protected from light at room temperature, and it was diluted to working concentrations in complete medium prior to use.

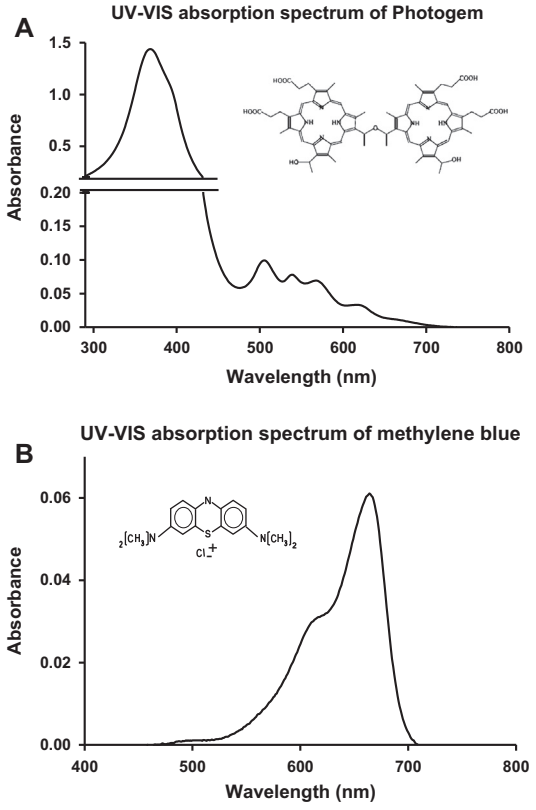

Fig. 1. Absorption spectra of Photogem (A) and methylene blue (B), obtained from diluted water solutions using a Lambda 1050 UV/Vis/NIR spectrophotometer (PerkinElmer, USA) at the Chemical Institute–UNESP Araraquara.

2.3. Light source

Both light sources (630 and 660 nm) consisted of a compact LED array-based illumination system with a homogeneous illumination area and a cooling device, composed of 48 LEDs with variable intensities (IrradLED® – biopdi, Sao Carlos, SP, Brazil). The distance between the LED and the plate allowed an even distribution of light on each well. The power density of the incident radiation was measured using a power meter (Coherent®, Santa Clara, CA, USA).

2.4. Photodynamic and cisplatin treatment

All three cell lines were seeded into 96-well plates (1×10^5 -cells/mL) and incubated overnight. In the cisplatin-only group, 8 different cisplatin concentrations were used (from 1.3 to 166 μM). The medium was changed after 6, 12 or 24 h, and cell viability was determined using the MTT assay after an additional 24 h as previously described [14].

Treatment groups are summarized in Table 1.

For the Photogem–photodynamic therapy (PG–PDT) group, the cells were untreated or exposed to 630 nm LEDs at 1.39 or 2.76 J/cm^2 after a 2 h incubation with different PG concentrations (from 0 to 8.5 μM; twofold dilutions). In the methylene blue-photodynamic therapy (MB–PDT) group, the cells were untreated or exposed to 660 nm LEDs at 1.29, 2.56, 5.11 or 12.9 J/cm^2 after a 20 min incubation with different MB concentrations (from 0 to 156.4 μM; twofold dilutions). After each PDT treatment, fresh media was added to the wells. The MTT assay was performed 24 h later.

In the combination group, cisplatin concentrations (from 1.3 to 41.6 μM) were administered 6, 12 or 24 h before or after MB–PDT or PG–PDT. The MTT assay was performed 24 h after each treatment.

For all MTT assays, 100 μL of MTT solution (0.5 mg/mL; in complete medium without serum) was added to each well and incubated for 3 h. The solution was then replaced by 100 μL of acidified isopropanol (4% HCl), and the absorbance was measured at 570 nm (BioTek Powerwave X, BioTek Instruments, Inc., Winooski, VT, USA). The percentage of cell viability was calculated by dividing the mean absorbance in each treatment group by the mean absorbance in the control group. The assay was performed as three independent quadruplicates.

2.5. Statistical analysis

Data were expressed as the mean plus standard deviation (SD) and were analyzed by one-way ANOVA with Tukey's post hoc test using GraphPad Prism® Version 5.01 software (GraphPad Software Inc., La Jolla, CA, USA). Differences were considered to be significant when $p < 0.05$. The acceptable coefficient of variation was less than or equal to 25%.

Table 1
Treatment groups for photodynamic therapy and cisplatin monotherapies and combination therapies.

Groups	(PS)	(Cisplatin)	Light dose
Methylene blue (MB)	0.0–156.4 μM	0	0
Photogem (PG)	0.0–8.5 μM	0	0
MB–PDT	0.0–78.2 μM	0	1.29–12.9 J/cm^2
PG–PDT	0.0–8.5 μM	0	1.39 and 2.76 J/cm^2
Cisplatin	0	0.0–166 μM	0
Cisplatin + MB–PDT	19.5 μM	1.3–41.6 μM	5.11 J/cm^2
Cisplatin + PG–PDT	0.5 μM	1.3–41.6 μM	2.76 J/cm^2

PS: photosensitizer; MB–PDT: methylene blue–mediated photodynamic therapy; PG–PDT: Photogem–mediated photodynamic therapy.

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