EVALUATION OF PHOTODYNAMIC THERAPY WITH LED IN TUMOR CELL ADHESION PROCESS

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Abstract:
Many cell adhesion molecules take part in cell-extracellular and intercellular matrix interactions of cancer. Photodynamic Therapy (PDT) has been shown to reduce cell adhesion and the ability of tumor cells to stick together. The purpose in this study is to evaluate cell-cell and cell-matrix adhesion in breast and laryngeal cancer lines. Cell-matrix and cell-cell adhesion analysis were performed with MCF-7 and HEp-2 cell lines, after PDT with different photosensitizers and Light Emitting Diode (LED) as light source. It was observed that, after PDT, both cell lines had lower levels of adherence not only for cell-matrix but also for cell-cell adhesion. Consequently, for both cell lines, PDT compromises cell adhesion process.

Keywords: Breast cancer, laryngeal cancer, LED therapy, photodamage.

Introduction

The study of new approaches and treatments for cancer has a range of focus. In order to comprehend different molecular characteristics, the evaluation of cell adhesion can elucidate some aspects, for instance, molecular capability of cell induction for a new biological behavior, which is able to change tumor adhesion [1].

Photodynamic Therapy (PDT) has a devastator role on cancer cells membranes and other cellular structures, affecting extracellular matrix (ECM) and adhesion components. According to Pazos *et al.* (2007) [2], PDT influences some ECM components, favoring immune system modulation. This therapy has been shown to influence cell adhesion [3] and consequently reducing the tumor metastasis process [4].

Photodamage caused by PDT influences cell adhesion to ECM components and between cells, indicating that the therapy modulates metastasis process because cell adhesion to ECM is key determinant in this process [5]. Although there is ample evidence that cell adhesion is vital to carcinogenic process, it is not completely elucidated the mechanisms of cell adhesion on tumor cell lines, especially for invasive and internal cancer.

This study aims to investigate and compare cell adhesion process in HEp-2 (human larynx carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines undergoing PDT with Photosan 3® (analogous to Photofrin®) and a precursor of the natural photosensitizer protoporphyrin IX, ALA® (5-aminolevulinic acid) for photosensitization, using Light Emitting Diode (LED) as light source.

Materials and Methods

Cell culture

HEp-2, human laryngeal cancer cell line, was obtained from Adolfo Lutz Institute, (São Paulo, Brazil). MCF-7, human breast adenocarcinoma cell line, was obtained from Banco de Células do Rio de Janeiro (Rio de Janeiro, Brazil). Both cell lines were routinely cultivated in flasks using Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Gibco®, USA) supplemented with 10% (vol/vol) Fetal Bovine Serum (FBS) (Life Technologies, Gibco®, USA) and 1% antibiotic–antimycotic (Life Technologies, Gibco®, USA), at 37ºC in humidified air atmosphere containing 5% of CO_2.

Photodynamic Therapy

The following groups were analyzed:

1. Control: Group free of any treatment (kept at room temperature with PBS, Phosphate Buffer Saline); 2. Control irradiated: Control group only submitted to LED irradiation; 3.PDT (3.1 Group incubated with ALA® 50μg/ml and subsequently irradiated; 3.2 Group incubated with Photosan 3® 100μg/ml and subsequently irradiated).

PDT cell groups were incubated with Photosan 3® and ALA® for 1 hour. After that, they were washed twice with PBS to remove the photosensitizer that had not been taken up by the cells and, subsequently, irradiated. After the therapy, cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Irradiation was performed in the dark with a portable LED, operating in continuous mode at a wavelength (λ) of 640±20nm, power of 70mW/cm² and energy density of 4.5 J/cm². LED equipment was calibrated using a laser power energy monitor (2W broad-band power/energy meter, Model 13 PEM 001/J).

Cell-Matrix Adhesion Assay

4, 24 and 48 hours after PDT, cells ($1x10^5$ cells/well) were washed with PBS, fixed with methanol and subsequently stained with crystal violet (0.1% diluted in 70% ethanol). They were washed with ultra-pure water and were extracted with Crystal Violet SDS (sodium dodecyl sulphate) at 1% in distilled water. The optical density of the plates was read at 570 nm in a microplate reader (SpectraCount- Packard®). Each experiment was run in triplicate.

Cell-Cell Adhesion Assay

HEp-2 cells ($1x10^3$ cells/well) were plated, forming a confluent monolayer in 96-wells plate. Other HEp-2 and MCF-7 cells were plated in Petri dish ($1x10^6$ cells/plate) and PDT was performed on them.

2 and 24 hours after PDT, cells were trypsinized, incubated with Calcein AM (Invitrogen® Carlsbad, CA, USA) (2μM/30min) and centrifuged at 2500 rpm for 3 minutes for twice. At the third time, the pellet was resuspended in DMEM 2% FBS.

MCF-7 and HEp-2 cells were deposited on the HEp-2 confluent monolayer. The determination of the cells attachment was evaluated at the end of each incubation period using fluorescence microscope (Leica 2 DMLB®).

Statistical analysis

Values obtained were converted to percentage and expressed in mean with standard deviation. Statistical calculations were done using Graph Prism® statistical data analysis software. Statistical differences were considered significant when $p<0.05$.

Results

Cell-Matrix Adhesion Assay

The results obtained in the evaluation of cell-matrix adhesion process demonstrated that the use of ALA® and Photosan 3® for photosensitization interferes the adhesive properties of cells under PDT.

For HEp-2 groups, the reduction of cell adhesion treated with Photosan 3® is very clear, however, after 48 hours, the adherence increases, as observed in figure 1.

For MCF-7 groups, the results obtained indicated that the use of ALA® is more significant after 4 and 24 hours treatment, while Photosan 3® is more effective after 48h PDT. However, both photosensitizers play a role in the adhesion properties of cells subjected to PDT, as indicated in figure 2.

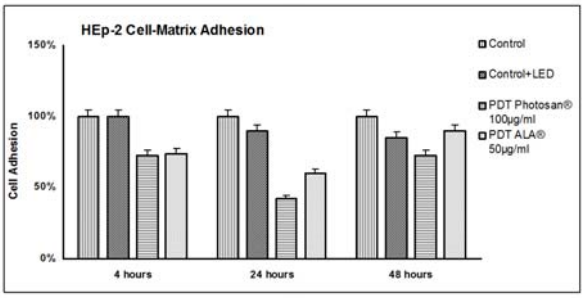

Figure 1: Cell-Matrix Adhesion Assay with HEp-2 Cells. Both photosensitizers reduced cellular adhesion, especially 24h after PDT. Photosan 3® had a greater adhesion reduction at all periods compared to the other groups.

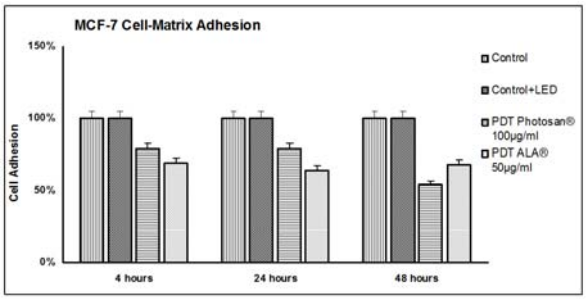

Figure 2: Cell-Matrix Adhesion Assay with MCF-7 Cells. After 4 and 24h, PDT ALA® indicated a greater adhesion reduction compared to the other groups. However, 48h after the therapy, cellular adhesion was lower when using Photosan 3® as photosensitizer.

Cell-Cell Adhesion Assay

For the study of the interaction between cells subjected to PDT and a culture of HEp-2 cells in monolayer, Calcein AM was used as indicator of cell viability. Cells were incubated for 2 and 24 hours to evaluate the ability of adhesion after PDT. The behavior of the control groups demonstrated an increasing of cells adhered to throughout the period analyzed. Cultures submitted to PDT had lower adhesion.

Figure 3 indicates that, for HEp-2 line, Photosan 3® lead to lower adhesion. However, figure 4 infers that, for MCF-7, ALA® lead to a greater cellular adhesion reduction, compared with the other groups.

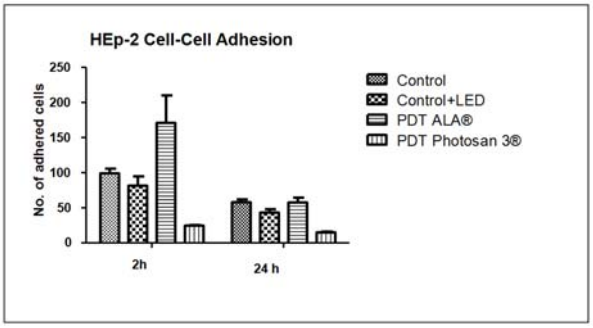

Figure 3: Cell-Cell Adhesion Assay with HEp-2 Cells. PDT Photosan 3® had a greater adhesion reduction compared to the other groups at 2 and 24h after PDT. As it can be observed, the adhesion was not reduced with

ALA®. This behavior is similar to the cell-matrix adhesion, indicated in figure 2.

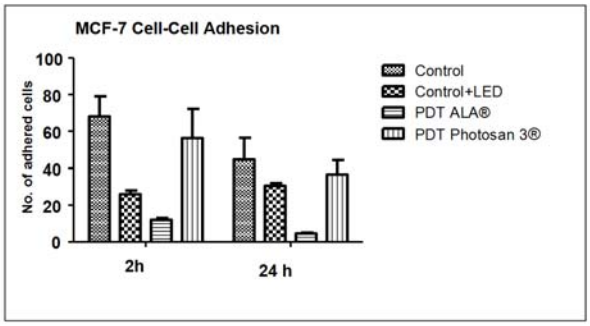

Figure 4: Cell-Cell Adhesion Assay with MCF-7 cells. PDT ALA® has a greater adhesion reduction compared to the other groups. Photosan 3® doesn't make cell-cell adhesion effect in this cell line, which means that ALA® is more indicated to PDT in HEp-2.

Discussion

The terminology cell adhesion represents direct contact between cells or between cells and ECM. Cell adhesion is essential for multicellular organisms' development, cell regulation, embryogenesis, morphogenesis and tissue regeneration [6].

Cell-Matrix Adhesion Assay

Cell-matrix binding is capable of signaling inside the cytoplasm, reorienting the cytoskeleton and changing cell behavior, stimulating cell proliferation [7].

Cell adhesion molecules (cadherins, selectins, integrins and immunoglobulins) play an important role in the growth and metastasis of cancer [8].

The impairment of adhesion between cells and ECM was observed in all PDT groups. Foultier *et al.* (1994) [9], using an hematoporphyrin derivative, also reported a decrease in tumor cell adhesion. The evaluation of HEp-2, as shown in figure 2 and 7, indicated that both PDT groups (ALA® and Photosan 3®) had great reduction of cell-matrix adhesion, compared to the control group. Group PDT Photosan 3® was the one with lower adhesion 4, 24 and 48 hours post treatment, suggesting efficiency of this photosensitizer for this tumor cell line.

For MCF-7, the graphic confirms that, after PDT with ALA®, the adherence was lower than for the other groups, however, 48 hours post therapy this behavior changes and it is observed less adhesion with Photosan 3®, as indicated in figure 3.

Cell-Cell Adhesion Assay

For this assay, analyzing HEp-2 adhesion on HEp-2 monolayer, a greater reduction in adhesion process was observed when using Photosan 3®, for both 2 and 24 hours groups after PDT, as indicated in figure 4, similar to the HEp-2 cell-matrix adhesion results. The statistical analysis confirmed that treatment changes adhesion and it is responsible for almost 55% of the total variance after 2 hours treatment. After 24 hours, PDT groups presented 29.9% of total variance ($p<0,0001$).

For MCF-7 adhesion to HEp-2 monolayer, the reduction was more significant when PDT was performed with ALA®, representing 60.68% of total variance ($p=0,0023$). This result indicates the same observed in cell-matrix adhesion assay, attesting PDT efficacy and different cell behavior to the photosensitizers. In general, reduction of adhesion to other cells capacity was demonstrated in figure 4.

These results were corroborated with Foultier et al (1994) [9], who used hematoporphyrin derivative and reported a decrease in colon cancer cell adhesion to a monolayer of endothelial cells.

Calcein AM, which indicates cell viability, passed through living cells membrane passively and was cleaved by intracellular esterases leading to a polar fluorescent derivative (calcein), which remains secluded in cytoplasm [10]. Calcein has demonstrated higher sensitivity, compared with Annexin V, to indicate apoptosis detection for early stage [11].

PDT demonstrated impact on adhesion, reducing the interaction between cells. The photosensitizers are directly related to the outcome of each cell line analyzed.

It is relevant that the Control irradiated group, for all the assays, obtained similar behavior to the control group, indicating that the therapy is functional only when the irradiation is associated with a specific photosensitizer.

Cell-cell adhesion assay demonstrated that MCF-7 and HEp-2 cells were not completely able to adhere to HEp-2 monolayer after PDT, indicating that the therapy altered cell-cell adhesion capability. ALA® and Photosan 3® influence cell-matrix adhesion, as HEp-2 and MCF-7 were not able to effectively adhere to the collagen matrix after the therapy, compared to control group. HEp-2 had greater reduction in cell-matrix adhesion when using Photosan 3®, while for MCF-7, the reduction was more significant when PDT was performed with ALA®. Finally, PDT with Photosan 3® is more efficient for HEp-2 cell adhesion and PDT with ALA®, for MCF-7 cells. Both photosensitizers induce damages that compromise cell adhesion, inhibiting cellular adhesion potential.

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