CYTOTOXICITY OF P-123-HYPERICIN PHOTODYNAMIC INACTIVATION ON HUMAN CELLS LINE

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ABSTRACT

Photodynamic inactivation (PDI) is a non-invasive technology, recognized since 1900, that uses a combination of a light source and a photosensitizer (PS), causing photochemical reactions. Hypericin (Hyp) is a napthodianthrone naturally isolated compound, with promising photodynamic properties by high penetration and absorption. A novel formulation was developed the encapsulated Hyp in P-123 copolymeric micelles (P-123-Hyp) formed at low copolymeric concentration, with the intention to improve the photodynamic characteristics of Hyp. Therefore, its necessary evaluate if PDI mediated P-123-Hyp cause cytotoxicity in cell line human. For this, assay was performed to evaluate the action of photoinactivation on keratinocytes *in vitro*. In cytotoxicity assay, Keratinocytes cells were incubated with concentrations of P-123-Hyp (0.09 - 50 µmol/L) for 2 h in the dark at 37°C in 0.5% CO_2, subsequently the cells were irradiated with LED at 27 J/cm^2. Then, post-irradiation keratinocytes were re-incubated for 24h in the dark at 37°C in 0.5% CO_2. The cell viability were evaluated with MTS, compared to untreated control. The results demonstrated non-cytotoxic in cells at concentrations ranging 0.78 - 50 µmol/L, and slight cytotoxicity in concentrations 0.09 - 0.39 µmol/L of P-123-Hyp. Thus, this study showed that the use of P-123-Hyp-PDI is safe promising treatment to several diseases.

KEY WORDS: keratinocytes; photodynamic inactivation; P-123-hypericin.

1 INTRODUCTION

Photodynamic inactivation (PDI) is a non-invasive technology, in which a combination of a light source and a photosensitizer (PS) causes photochemical reactions that generates reactive oxygen species (ROS) and promotes high microbial cytotoxicity and therefore cell killing (CASTANO; DEMIDOVA; HAMBLIN, 2004; LÓPEZ-MARÍN; MULET; RODRÍGUEZ, 2018). This therapy has been recognized since 1900, after the researchers Oscaar Raab and Herman Von Tappeiner verified the cell death by the interaction of light with a PS. The PDI has been implemented as a low cost treatment with satisfactory results for treatment of superficial infections, such as onychomycosis cases, and even neoplastic cells (MEGNA et. al, 2017).

Hypericin (Hyp) is a napthodianthrone naturally compound isolated from species of plants of the genus *Hypericum*, known as St. John's Wort. The Hyp alone was used for common illness such as headaches and rheumatism in the history of folk medicine, and in 1930 recognized as a PS with photodynamic properties by high penetration and absorption (DURAN; SONG, 1986). Since then, Hyp has been implemented in PDI as a treatment for skin diseases, tumors, cancer and infectious diseases (KARIOTI; BILIA, 2010).

The use of nanotechnology promove a novel formulation was developed the encapsulated Hyp in P-123 copolymeric micelles (P-123-Hyp) formed at low copolymeric concentration, with the intention to improve the photodynamic characteristics of Hyp (GONÇALVES, R.S et al, 2017). In this context, the PDI mediated P-123-Hyp to promising for the different pathologies. The activity of PDI may to cause irreversible damage to

microorganism or human cell, target dependent. In this sense, is important studies of the cytotoxicity of the photoinactivation with P-123-Hyp (P-123-Hyp-PDI) in human cell line.

2 MATERIALS AND METHODS

Photosensitizer, P-123-Hyp was synthetized and encapsulated in accordance with Sakita, et. al (2019). To cytotoxicity assay, Keratinocytes (HaCat) were cultivated in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) contained 10 % fetal bovine serum (Gibco/Invitrogen) and 100 units of penicillin and 0.01 mg streptomycin (Sigma-Aldrich) at 37°C in 0.5% CO_2 for 24 h. Then, the cells were removed using 0.05 % trypsin-ethylene-diamine-tetra-acetic acid (EDTA, Gibco/Invitrogen) solution, and were subcultured in a 96-well plate at a concentration of $2x10^5$ cells/mL and allowed to adhere for 24 h, reaching 80% confluency (FARIA et. al, 2017). After, the keratinocytes were incubated for 2 h with various concentrations of P-123-Hyp (0.09 - 50 µmol/L) protect from light at 37°C in 0.5% CO_2, and the plate was irradiated with LED at 27 J/cm^2. Post-irradiation, the cells were re-incubated for 24h in the dark at 37°C in 0.5% CO_2. Three controls were included: positive control ((PC) unirradiated cells with DMEM), light control ((LC) cells with DMEM irradiated with 27 J/cm^2), dark control ((DC) cells with Hyp without irradiance).

To evaluated the cell viability, the cells were washed with phosphate buffered saline (PBS - pH 7.4) and 100 µl of a solution MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) was added, incubated protected from light at 37°C in 0.5% CO_2 for 3h (WIELGUS et. al, 2007). The mitochondrial activity was determined for absorbance at 492 nm. The values of cytotoxicity 24 h post-P123-Hyp-PDI on HaCat cells were evaluated compared with PC (100% of viability). Results were analyzed in accordance with ISO-standard 10993-5, in which inhibition of < 30% counts is non-cytotoxic, 30 – 50 % as slight, 50 – 70 % as moderate and > 70 % as very cytotoxic ISO-standard 10993-5, (2009).

3 RESULTS AND DISCUSSION

P-123-Hyp-PDI demonstrated non-cytotoxic in the concentrations ranging 0.78-50 µmol/L, with cellular toxicity below 30%. On the other hand, in 0.09-0.39 µmol/L of encapsulated Hyp presented a slight cytotoxicity, with of 44% cell death (Table 1). All concentrations tested of P-123-Hyp in the dark (DC) and light control had not cytotoxicity, data not show.

Table 1. Cytotoxicity 24 h post-P-123-Hyp-PDI on HaCat cells.

P-123-Hyp µmol/L	% Cytotoxicity Mean±SD
50	18±14
25	15±4
12.5	27±4
6.25	20±10
3.12	25±2
1.56	25±2
0.78	26±10
0.39	40±8
0.19	44±3
0.09	44±3

Despite the variation in cell viability between P-123-Hyp-PDI concentrations, it can be considered safe for use in human cells, because the results show absence or low

cytotoxicity. In addition, the low toxicity evaluated at concentrations below 0.78 µmol / L is in agreement with other authors such as PELLISSARI et. al, 2016.

Study *in vitro* performed by REJUSTA et. al, 2012 demonstrated that keratinocytes were preserved with cell viability > 90% in concentrations up to 1 µmol of Hyp with light dose up to 37 J/cm^2. Additionally, Hyp (2 mg/mL, 75 J/cm^2) alone was evaluated *in vivo* in the nonmelanoma skin cancer model, and no toxicity was observed in normal cells (KACEROSKÁ et. al, 2008). In this study, we used Hyp incorporated in micelles (P-123), which did not impair cell viability. Jambeck et. al (2014) studied liposomes as carriers of Hyp, and demonstrated results similar to ours, where cytotoxicity is inversely proportional to Hyp concentration. The authors attribute the toxicity to better cell permeation of Hyp, probably due to the lower aggregation of this compound at low concentrations.

4 FINAL CONSIDERATIONS

This study showed that P-123-Hyp-PDI not is cytotoxicity in HaCat cells. Low concentrations encapsulated Hyp cause slight cytotoxicity, but according in literature, high concentrations was used to treatment *in vivo* with higher doses of irradiation, and not demonstrated cytotoxicity.

Therefore, the photoinactivation mediated by P-123-Hyp shows low cytotoxicity, can be promising treatment safe for the application to skin diseases, tumors, cancer, infectious diseases, such as superficial and subcutaneous mycoses.

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