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Application of aluminum chloride phthalocyanine-loaded solid lipid nanoparticles for photodynamic inactivation of melanoma cells

Patrícia L. Goto[a], Marigilson P. Siqueira-Moura[b], Antonio C. Tedesco[a,c,*]

[a] Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Ciências Farmacêuticas, Avenida do Café, s/n°, Ribeirão Preto, SP, 14040-903, Brazil
[b] Universidade Federal do Vale do São Francisco, Colegiado Acadêmico de Ciências Farmacêuticas, Avenida José de Sá Maniçoba, s/n°, Centro, Petrolina, PE, 56304-205, Brazil
[c] Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Departamento de Química, Avenida Bandeirantes, 3900, Ribeirão Preto, SP, 14040-901, Brazil

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ABSTRACT

Cutaneous melanoma is the most aggressive skin cancer and is particularly resistant to current therapeutic approaches. Photodynamic therapy (PDT) is a well-established photoprocess that is employed to treat some cancers, including non-melanoma skin cancer. Aluminum chloride phthalocyanine (ClAlPc) is used as a photosensitizer in PDT; however, its high hydrophobicity hampers its photodynamic activity under physiological conditions. The aim of this study was to produce solid lipid nanoparticles (SLN) containing ClAlPc using the direct emulsification method. ClAlPc-loaded SLNs (ClAlPc/SLNs) were characterized according to their particle size and distribution, zeta potential, morphology, encapsulation efficiency, stability, and phototoxic action *in vitro* in B16-F10 melanoma cells. ClAlPc/SLN had a mean diameter between 100 and 200 nm, homogeneous size distribution (polydispersity index <0.3), negative zeta potential, and spherical morphology. The encapsulation efficiency was approximately 100%. The lipid crystallinity was investigated using X-ray diffraction and differential scanning calorimetry and indicated that ClAlPc was integrated into the SLN matrix. The ClAlPc/SLN formulations maintained their physicochemical stability without expelling the drug over a 24-month period. Compared to free ClAlPc, ClAlPc/SLN exerted outstanding phototoxicity effects *in vitro* against melanoma cells. Therefore, our results demonstrated that the ClAlPc/SLN described in the current study has the potential for use in further preclinical and clinical trials in PDT for melanoma treatment.

1. Introduction

The incidence of skin cancer has increased worldwide and has affected public health globally (Gandhi and Kampp, 2015; Gordon, 2013). Melanoma is the most aggressive type of skin cancer. Although it represents only 4% of all new skin cancer cases, this malignancy is highly metastatic and accounts for approximately 85% of skin cancer deaths (Gordon, 2013; Kolk et al., 2014). The pathogenesis of this cutaneous carcinoma is multifactorial; nevertheless, exposure to UV radiation is the main risk factor predisposing patients to the disease (Gordon, 2013; Lazareth, 2013; Narayanan et al., 2010). In general, melanoma affects the melanocytes, which are responsible for producing melanin, the pigment that protects the skin against the harmful effects of the sun. Because melanin absorbs UV radiation, it works as a light shield (Mundra et al., 2015; Narayanan et al., 2010). When detected in the early stages, the only curative treatment for cutaneous melanoma is surgical excision of the tumor, which does not always

Abbreviations: PDT, photodynamic therapy; ClAlPc, aluminum chloride phthalocyanine; SLN, solid lipid nanoparticles; ClAlPc/SLN, ClAlPc-loaded SLN; ROS, reactive oxygen species; HLB, hydrophilic lipophilic balance; PCS, photon correlation spectroscopy; PdI, polydispersity index; PS, photosensitizer drug; EE, encapsulation efficiency; TEM, transmission electron microscopy; AFM, atomic force microscopy; ANOVA, analysis of variance; CD, crystallinity degree; DMEM, Dulbecco Eagle's minimum essential medium; DSC, differential scanning calorimetry; Et, ethanol; FBS, fetal bovine serum; MTT, 4,5-dimethylthiazol-2-yl)2,5-dyphenyl-tetrazolium bromide; NIR, near infrared; PBS, phosphate-buffered saline; RCF, relative centrifugal force; SD, standard deviation; STEP, space and time resolved extinction profiles; XRD, X-ray diffraction.

* Correspondence to: Laboratório de Fotobiologia e Fotomedicina (FFCLRP) Universidade de São Paulo (USP) Av. dos Bandeirantes 3900, 14040-901, Vila Monte Alegre, Ribeirão Preto, SP, Brazil.
E-mail address: atedesco@usp.br (A.C. Tedesco).

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prevent melanoma progression. Immunotherapy, gene therapy, chemotherapy, and radiotherapy have been used as adjuvant treatments (Ingraffea, 2013; Monge-Fuentes et al., 2014). However, these therapies do not always provide significant and effective results. In addition, the drugs are highly toxic, and drug resistance may emerge after a short period of treatment. Considering the incidence, metastasis, mortality rate, and therapeutic resistance of melanoma, developing effective therapeutic approaches has become crucial (Girotti et al., 2014; Kawczyk-Krupka et al., 2013; Vera et al., 2015).

In this scenario, photodynamic therapy (PDT), which has already been established as a local therapy for several cancers and skin disorders (Baldea and Filip, 2012; Davids and Kleemann, 2011; Kawczyk-Krupka et al., 2013; Kolk et al., 2014; Sparsa et al., 2013), might be a potential tool for melanoma treatment. PDT combines three essential components: (i) the administration of the photosensitizer (PS), followed by (ii) PS photoactivation with visible light at an appropriate wavelength and (iii) PS interaction with molecular oxygen, generating singlet oxygen and reactive oxygen species (ROS), which lead to cell death. Furthermore, PDT can be applied several times, alone or in combination with other modalities, resulting in low systemic toxicity and excellent cosmetic results (Agostinis et al., 2011; Babilas et al., 2010). Over the last decades, *in vivo* and *in vitro* studies (Davids and Kleemann, 2011; Mroz et al., 2010; Sharma and Davids, 2012) investigating the role of PDT in cutaneous melanoma treatment concluded that pigmented tumors were resistant to PDT. Two possible explanations for this fact have been proposed: (i) melanin competed with PS for light absorption, and (ii) melanin exhibited high antioxidant power.

Aluminum chloride phthalocyanine (ClAlPc) is a PS that contains aluminum (III) as a central metal ion, which can enhance the photophysical and photochemical properties of this phthalocyanine (Siqueira-Moura et al., 2013). ClAlPc presents a high molar absorption coefficient between 600 and 800 nm, also known as the "optical window" of biological tissues, in which light with enough energy to produce ROS can reach the deeper skin layers with little interference from the body's endogenous chromophores, such as melanin (Debele et al., 2015; Ficheux, 2009; Lucky et al., 2015; Plaetzer et al., 2008). In fact, it has been shown that ClAlPc is effective in treating pigmented diseases, and it has been suggested that ClAlPc could overcome the typical PS–melanin competition (Allison and Sibata, 2010; Idowu and Nyokong, 2007). ClAlPc has excellent photochemical and photodynamic activity, however, like most phthalocyanines, ClAlPc is insoluble in water, which limits its bioavailability and its administration in a physiological environment. In an aqueous medium, hydrophobic molecules tend to aggregate, which directly affects the photophysical and photochemical properties of the PS and dramatically reduces the photodynamic activity of ClAlPc (Chatterjee et al., 2008), requiring association to specific drug nanocarriers or conjugation of the PS with hydrophilic molecules for clinical use. The use of nanotechnology-based drug delivery systems could overcome these shortcomings, and provide additional advantages, such as (a) promotion of controlled PS release, (b) protection from drug degradation, (c) reduced amounts of PS required to achieve therapeutic effects (hence diminishing the toxicity effects), and (d) increased PS stability and bioavailability (Bechet et al., 2008; Lucky et al., 2015; Paszko et al., 2011).

Solid lipid nanoparticles (SLN) have emerged as promising drug delivery systems (Mehnert and Mader, 2001). First developed in the 1990s, these solid colloidal particles consist of a solid lipophilic matrix at body temperature, in which biologically active substances can be dissolved or entrapped (Müller et al., 2011; Simões et al., 2015). They have a mean particle size in the submicron range between 50 and 1000 nm (Müller et al., 2011; Pardeike et al., 2009). In addition, SLN have many advantages when compared to other colloidal carriers, such as avoidance of organic solvents, low cost materials, drug release profile controlled by modification of the solid matrix, improved stability profile, and the possibility of large scale production (Kakadia and Conway, 2014; Mehnert and Mader, 2001). This colloidal system can be administered *via* different routes, but its topical application on the skin has attracted much attention because SLN strongly adhere to the stratum corneum, to form a flexible lipid film on the skin. This occlusion effect keeps the skin hydrated and improves the percutaneous absorption of the drug (Souto et al., 2006), aiding the treatment of cutaneous melanoma. In addition, the nanosize and the lipophilic composition of this colloidal system contribute to its preferred and passive accumulation in the tumor tissue due to the enhanced permeability retention effect (enhanced vascular permeability and poor lymphatic drainage) of the abnormal tissue (Debele et al., 2015; Iyer et al., 2006; Mundra et al., 2015).

In this paper we described the preparation and characterization of SLN as a controlled drug delivery system for ClAlPc using the direct emulsification method and tested their phototoxicity effect in B16-F10 cells *in vitro* to investigate their efficacy in PDT for melanoma treatment.

2. Materials and methods

2.1. Materials

The solid lipid glyceryl behenate (Compritol 888 CG ATO, melting range 69–74 °C) was a gift from Brasquim (Porto Alegre, RS, Brazil). The solid lipid stearic acid (melting range 67–72 °C), aluminum chloride phthalocyanine (ClAlPc, purity = 85%), phosphate-buffered saline (PBS, pH 7.2–7.4), trypan blue, and 4,5-dimethylthiazol-2-yl)2,5-dyphenyl-tetrazolium bromide (MTT) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Sorbitan isostearate (Hydrophilic Lipophilic Balance = 4.7) and Polyoxyethylene-40 hydrogenated castor oil (HLB = 14.1) were kindly supplied from Croda do Brasil Ltda (Campinas, SP, Brazil). The murine fibroblast cell lineage (NIH-3T3-CRL1658) and the murine melanoma cells (B16-F10–CRL 6475) were supplied by ATCC (Virginia, USA). Dulbecco Eagle's minimum essential medium (DMEM), RPMI-1640 with and without phenol red, and trypsin – EDTA (0.25%) were acquired from Gibco (New York, USA). Fetal bovine serum (FBS) was obtained from Cultilab (São Paulo, SP, Brazil). Methanol, chloroform, ethanol (≥99.5%), and dimethyl sulfoxide were purchased from J.T. Baker (Mallinckrodt Baker, Phillipsburg, USA). All of the other reagents were of analytical grade and were used as supplied. Ultrapure water, obtained using the Direct-Q Water Purification System (Millipore, Darmstadt, Germany), was used to prepare the colloidal dispersions.

2.2. Ternary phase diagram

To obtain SLN with the best features and component ratio (Anton et al., 2007), ternary phase diagrams were constructed for the solid lipid, the surfactant blend, and water. First, the non-ionic surfactants sorbitan isostearate and polyoxyethylene-40 hydrogenated castor oil were mixed at ratio of 33:67 (w/w), as reported previously by Goto et al. (2012), result in a mixture of surfactants with an $HLB_{final} = 11$. This mixture should provide oil in water dispersions. Different ratios of the surfactant blend were used in the final SLN composition. The same points of the ternary diagram were reproduced for each solid lipid (glyceryl behenate or stearic acid). The solid lipid and the surfactant blend were added at weight percentages ranging from 5 to 20% (w/w). Finally, the aqueous phase (ultrapure water ranging from 60% to 100%, w/w) was added to complete the dispersion to 100%. After 24 h, the resulting