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Development, characterization, and photocytotoxicity assessment on human melanoma of chloroaluminum phthalocyanine nanocapsules

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ABSTRACT

In this work we have developed nanocapsules containing chloroaluminum phthalocyanine (ClAlPc) and assessed their phototoxic action on WM1552C, WM278, and WM1617 human melanoma cell lines. The ClAlPc-loaded nanocapsules were prepared by the nanoprecipitation method and optimized by means of a 2^3 full factorial design. The ClAlPc nanocapsules were characterized by particle size and distribution, zeta potential, morphology, encapsulation efficiency, singlet oxygen production, stability, and phototoxic action on melanoma cells. Both the development and optimization studies revealed that stable colloidal formulations could be obtained by using 1.75% (w/v) soybean lecithin, 1.25% (w/v) Poloxamer 188, 2.5% (v/v) soybean oil, and 0.75% (w/v) poly(D,L-lactide-co-glycolide). The nanocapsules had a mean diameter of 230 nm, homogeneous size distribution (polydispersity index<0.3), and negative zeta potential (about −30 mV). Their morphology was spherical, with evident polymer membrane coating droplet. The encapsulation efficiency was 70%, as expected for hydrophobic drugs, and the nanoencapsulated ClAlPc was able to produce high singlet oxygen quantum yield. ClAlPc nanocapsules exhibited good physical stability over a 12-month period. WM1552C primary melanoma cells were more sensitive ($p<0.05$) to the phototoxic effect elicited by ClAlPc nanocapsules (0.3 μg ml^{-1}) under light irradiation at 20 mJ cm^{-2}. On the other hand, the cell survival percentage for all the melanoma cell lines treated with the highest light dose (150 mJ cm^{-2}) was lower than 10%. In summary, ClAlPc nanoencapsulation could enable application of this hydrophobic photosensitizer in the treatment of malignant melanoma with the use of both low sensitizer drug concentration and light dose.

1. Introduction

Photodynamic therapy (PDT) is a well-established photochemotherapeutic clinical treatment that can be applied in the case of several dermatological diseases, including superficial and localized tumors such as Bowen's disease, actinic keratosis, and basal cell carcinoma, among others [1–3]. The PDT protocol usually involves the administration of a non-toxic dye known as the photosensitizer (PS) drug, followed by irradiation of the lesion with visible light (typically long wavelength red light) after some time. In the presence of oxygen, this procedure results in the photogeneration of cytotoxic species that can damage cellular constituents, culminating in cell death and tissue destruction [3–5].

PS activation upon absorption of visible light with appropriate energy takes it from its ground state (^{1}PS) to an excited singlet state (^{1}PS*), and the PS molecule may undergo spin conversion to an isoenergetic level known as triplet state (^{3}PS*). Thereafter, ^{3}PS* can react directly with a substrate (type I photochemical reaction) by proton or electron transfer, to form radicals or radical ions. Alternatively, it can transfer energy directly to ground state triplet oxygen (type II photochemical reaction), to produce excited singlet state oxygen ($^1O_2$) which is one of the most damaging species generated during PDT [3,6]. The singlet oxygen produced by the type II reaction is a key cytotoxic agent that is able to directly kill neoplastic cells by induction of apoptosis and/or necrosis [4].

PDT has been successfully used in the treatment of non-melanoma skin cancer, especially basal cell carcinoma [3]. PDT has not been designed to treat melanoma skin cancer, and poor results have been reported for the treatment of cutaneous melanoma with “first-generation” PSs, which absorb at wavelengths lower than 600 nm; e.g., porphyrin.

Melanoma is the rarest form of skin cancer and accounts for 4% of all the types of this disease. However, it is the most aggressive cutaneous cancer and is responsible for about 75% of all the deaths from such cancers, and its incidence is rapidly increasing worldwide [7–9]. The development and progression mechanism of malignant melanoma can be better realized considering the sequence of the following five events: i) inherited or simple acquired nevi with structurally normal melanocytes, ii) dysplastic nevus with structural and architectural atypia, iii) early radial growth phase (RGP) primary

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melanoma, iv) advanced vertical growth phase (VGP) primary melanoma, and v) metastatic melanoma [10–12]. Melanoma is known for its resistance to all the classical treatments used against cancer (chemotherapy and radiotherapy). Although research has led to significant progress in the understanding of melanoma biology and genetics, no effective treatment is currently available yet [7,12,13].

The photoresistance of melanoma to first-generation photoactive drugs could be due to the melanin pigment which scatters visible light at lower level [14]. However, PS drugs that absorb at wavelengths longer than 630 nm could avoid the light absorption scattering process caused by this endogenous pigment, which has a maximum light absorption around 530 nm. Moreover, red light allows deeper tissue penetration with higher tumor specificity [12,14]. In this context, "second-generation" PS drugs belonging to the class of metallophthalocyanines have emerged as more appropriate PSs for application to pigmented lesions, since these PS drugs present typical maximum absorption located at 670–680 nm in the red region of the visible spectrum [15–17].

Chloroaluminum phthalocyanine (ClAlPc), in particular, is a PS molecule consisting of a tetrapyrrolic macrocycle and a central metal ion (Al3 +) (Fig. 1). The incorporation of a diamagnetic metal; e.g., aluminum, into the macrocycle provides the phthalocyanine with the most favorable photophysical and photochemical properties for application in PDT, such as high triplet state quantum yields and long triplet lifetimes, as well as high singlet oxygen production yield [15,16,18]. However, ClAlPc is characterized by its high hydrophobicity which results in the formation of photochemically inactive aggregates in aqueous solution due to electronic interactions between the rings of two or more molecules. Therefore, the poor ClAlPc solubility often prevents its direct application in the target biological tissue [19]. In order to overcome this limitation, several encapsulation strategies have been used for improvement of the solubility and bioavailability of lipophilic compounds including oily core-based nanoparticulate formulations [17,20–25].

Nanocapsules are defined as submicronic vesicular systems composed of an oily core surrounded by a thin polymeric membrane [24–26]. Their advantages as a drug delivery system include high efficiency regarding the encapsulation of lipophilic drugs, drug polymeric shell protection against chemical degradation, reduction of tissue irritation due to the polymeric membrane, low polymer content as compared to nanospheres, and drug entrapment into a central cavity (which may avoid a burst effect), just to mention a few benefits [23,24,26,27]. The hydrophobic drug can be entrapped in the central oil core and/or adsorb onto the polymeric membrane of the nanocapsules [27].

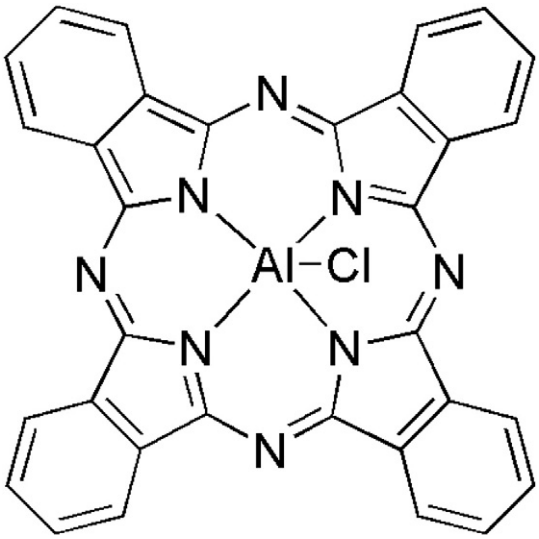

Fig. 1. Chemical structure of chloroaluminum phthalocyanine ($C_{32}H_{16}AlClN_8$).

Photosensitizer-loaded biodegradable nanocarriers have been studied as potential nanocarriers for utilization in PDT [3,28,29]. In this sense, the aim of this work was to design and optimize colloidal polymer particles containing a hydrophobic photosensitizer (ClAlPc) with a view to their application in PDT against melanoma skin cancer. The designed colloidal formulation was optimized by means of a 2^3 factorial study, in which the influence of the three main factors from nanocapsules formulation (i.e., lipophilic and hydrophilic surfactants, and oily core) was evaluated on average diameter of particles, size distribution, and zeta potential. Furthermore, the most stable ClAlPc nanocapsule formulation was evaluated in terms of its potential phototoxicity against human melanoma cells at different stages of tumor progression.

2. Materials and methods

2.1. Materials

Aluminum phthalocyanine chloride (85% pure), Poly(D,L-lactide-*co*-glycolide) (PLGA) polymer (50:50), Poly (ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) EO_{76}-PO_{29}-EO_{76} (Pluronic F-68®), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and soybean oil were purchased from Sigma (Sigma-Aldrich co., St. Louis, MO, USA). Miglyol 812 oil was provided by Hulls (Puteaux, France). Soybean lecithin (Lipoïd S 100®) was acquired from Lipoïd GmbH (Ludwigshafen, Germany). All the other chemicals were analytical grade. Ultrapure water was obtained from an E-pure apparatus (Barnstead, Iowa, USA).

2.2. Preparation and optimization of nanocapsules containing ClAlPc

ClAlPc-loaded nanocapsules were prepared using preformed polymer nanoprecipitation method as described by Fessi et al. [30]. This method allows for the spontaneous formation of nanometric oily droplets surrounded by a polymeric membrane. Typically, an exact quantity of the PLGA polymer (0.75% w/v), ClAlPc (0.5 mg), oil (2.5% v/v), and lecithin (phosphatidylcholine from soybean) were weighted and dissolved in acetone at 40 °C. This organic phase was poured into an aqueous Pluronic F-68 solution under moderate stirring, followed by organic solvent removal by evaporation under reduced pressure at 40 °C and concentration of the aqueous phase to 10 ml. The formulations were stored at 4 ± 2 °C.

2.2.1. Experimental design

In order to optimize the preparation of the ClAlPc-loaded nanocapsules, the experiments were performed by the nanoprecipitation method using a 2^3 full factorial design. The three independent variables were lipophilic surfactant concentration (Lipoïd S 100®), hydrophilic surfactant concentration (Pluronic F-68®, also known as Poloxamer 188), and oil type (Miglyol 812 or soybean oil). The selected variables were taken at low and high levels, and the values and coded units are presented in Table 1. The dependent variables of interest were particle size, polydispersity index (PdI), and zeta potential (or ζ potential). The design required a total of eight ClAlPc-loaded nanocapsules

Table 1
Experimental conditions of 2^3 factorial design parameters for nanocapsules formulation.

Variables (factors)	Coded units	Levels	
		Low	High
Lipophilic surfactant (%)[a]	*A*	1.25	1.75
Hydrophilic surfactant (%)[a]	*B*	1.25	1.75
Type of oil	*C*	Miglyol 812	Soybean oil

[a] % w/v.