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Evaluation of nanoparticles loaded with benzopsoralen in rat peritoneal exudate cells

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Abstract

Psoralens are widely used for the treatment of hyperproliferative skin disease. In this work, we prepared nanoparticles (NP) containing a benzopsoralen (3-ethoxy carbonyl-2*H*-benzofuro[3,2-*f*]-1-benzopiran-2-one) by the solvent evaporation technique. We evaluated important NP parameters such as particle size, drug encapsulation efficiency, effect of the encapsulation process over the drug's photochemistry, zeta potential, external morphology, and *in vitro* release behavior. We also investigated the nanoparticle as a drug delivery system (DDS), as well as its target delivery to the action site, which is a very important parameter to increase the therapeutic use of psoralens and to reduce their side effects. The uptake of benzopsoralen-loaded PLGA nanoparticles by different kinds of cells found in rat peritoneal exudates was also studied. The photodamage promoted by irradiation with UV light revealed morphological characteristics of cell damage such as cytoplasmic vesiculation, mitochondrial damage, and swelling of both the granular endoplasmatic reticulum and nuclear membrane. This encapsulation method maintained the drug's properties and improved drug delivery to the target cell.

Keywords: Benzopsoralen; Exudates peritoneal cell; Nanoparticle; PUVA

1. Introduction

The association of psoralens with ultraviolet A (UVA) irradiation (320–400 nm) is currently being employed in dermatology (orally or topically) (Makki et al., 1996; Pires et al., 2004). This combination is known as PUVA therapy (Lysenko et al., 2000; Machado et al., 2001; Canton et al., 2002; Roop et al., 2004). It has already been established that macrophages and neutrophils can be the initial target of PUVA therapy (Edelson, 1990; Okamoto et al., 1993). This treatment is effective against diseases such as vitiligo, psoriasis, mycosis fungoides, and atopic eczema, among others (Saiad et al., 1997; Tokura, 1999; Mariano et al., 2002; Tatchen et al., 2004). Unfortunately, most photosensitizing chemicals are also phototoxic to the skin, and skin contact with these molecules in the presence of UV irradiation results in sunburn, erythematic, and eventual edema (Middelkamp-Hup et al., 2004; Roop et al., 2004), thus limiting the use of PUVA therapy for the treatment of skin disorders (Lindelöf et al., 1999; Middelkamp-Hup et al., 2004). Therefore, in recent years, several highly photoreactive molecules have been synthesized aiming at developing new photochemotherapeutic drugs with fewer side effects (Machado et al., 2001; Oliveira et al., 2003; Roop et al., 2004). In this study, we evaluated the effectiveness of the compound 3-ethoxycarbonyl-2*H*-benzofuro-[3,2-*f*]-1-benzopyran-2-one (psoralen A), synthesized and kindly supplied by Oliveira-Campos (Scheme 1B) (Oliveira et al., 2003), for PUVA therapy. In this molecule, the presence of a benzene ring fused to the furan and the existence of bulky or electron-withdrawing substituents in the pyrone ring should inhibit the formation of adducts between psoralens and DNA (Machado et al., 2001; Oliveira et al., 2003). The introduction of an ester group into a benzopsoralen can furnish efficient photosensitizer derivatives, leading to high yield of singlet oxygen production (Machado et al., 2001; Llano et al., 2003). The photophysical properties of this compound have been recently investigated (Machado et al., 2001; Oliveira et al., 2003), and it has been shown that they can photochemically sensitize the generation of singlet oxygen with a quantum efficiency

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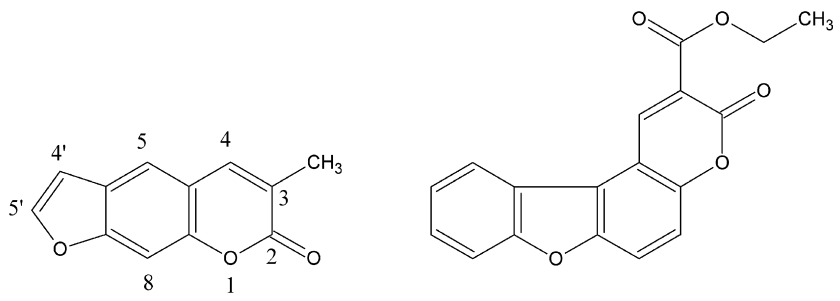

(A) (B)

Scheme 1. Representation of a typical psoralen (A) and psoralen A (3-ethoxy carbonyl-2*H*-benzofuro[3,2-*f*]-1-benzoyiran-2-one) (B).

approaching unity (Machado et al., 2001), contrary to other compounds (8-methoxypsoralen, 5-methoxypsoralen, trimethylpsoralen) (Carter et al., 1973; Cohen et al., 1981; Collins et al., 1996; Legat et al., 2001; Man et al., 2004) usually employed in PUVA therapy.

A drug delivery system (DDS) may be used to enhance the action of PUVA therapy, this is because this approach might help reduce undesirable side effects caused by psoralen, which will be directed to the target cells; *i.e.*, neutrophils and macrophages. Special interest has been focused on the use of particles prepared from polyesters like poly(DL-lactide-*co*-glycolide) (PLGA), because of their biocompatibility and biodegradability through natural pathways (Lacasse et al., 1998; Alleman et al., 1998). Recent works have described the influence of particle size and incubation time on the phagocytosis process (Panyam et al., 2003, 2004; Gomes et al., 2005), for the majority of the particles to became completely phagocyted by cells. PLGA microparticles were only phagocyted by macrophages in rat peritoneal exudate cells (Gomes et al., 2006).

In this work, we report the photophysical properties, particle size, zeta potential, and drug release profile of benzopsoralen-loaded NP. We also describe the effect of psoralen A on cells exposed to irradiation by UVA light. One of the advantages in using a DDS to carry drugs is the fact that a higher concentration of the drug can be administered. In some cases, the use of a drug in solution in the same drug concentration as used in DDS is not desirable because of this toxic effect or indiscriminate distribution through the body. The evaluation of the photobiological effects of PUVA in a DDS in the initial target cells of PUVA therapy (macrophage and neutrophyl) was carried out by employing the rat peritoneal exudate cells model. Transmission electron microscopy (TEM) helped demonstrate that the drug was entrapped in the NP and that it was able to successfully promote efficient photodamage in the target cells of PUVA therapy.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-*co*-glycolic acid) (PLGA, 50:50, Mw 17 kDa) was obtained from Sigma Chemical, Inc. (St. Louis, USA); poly(vinyl alcohol) (PVA, 13–23 kDa, 87–89% hydrolyzed) was supplied by Aldrich, (Milwaukee, USA); dichloromethane (analytical grade) was supplied by VETEC (Rio de Janeiro, Brazil). The compound 3-ethoxy carbonyl-2*H*-benzofuro[3,2-*f*]-1-benzopiran-2-one (psoralen A) was synthesized and supplied by Oliveira-Campos, from Minho University. All other chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of the polymeric psoralen nanoparticle

Nanoparticles (NP) containing psoralen A were produced by the solvent evaporation technique, as described by Gomes et al. (2005). Typically, the organic phase consisted of 0.1 g of the PLGA 50:50 polymer and 10 μM psoralen A dissolved in 10 mL of CH_2Cl_2. The dispersed phase was dropped into the homogeneous aqueous phase (100 mL of an aqueous phase containing 1% (w/v) of 88% hydrolyzed PVA as dispersing agent) under ice cooling, with stirring at 15,000 rpm for 3 min, using an Ultraturrax model T25 equipped with an S25N dispersing tool (IKA Laboratory Technology, Staufen, Germany). Further solvent evaporation was carried out by gentle magnetic stirring at room temperature, for a period of 3–5 h. NP was recovered by centrifugation for 5 min, at 10,000 rpm and 4 °C. They were then washed (three times) with distilled water and lyophilized (Labconco®, USA). NP without psoralen A was prepared by the same procedure. Dried NP was stored in a sealed glass vial and placed in a desiccator kept at 4 °C.

2.2.2. Morphology of nanoparticles: SEM analysis

Surface morphology of the nanoparticles was evaluated by scanning electron microscopy (SEM). Each sample was mounted on aluminum stubs and coated with a 50 nm gold coating under argon atmosphere, because the loaded NP lacks electrical conductivity. The diameter of the NP on the SEM was then measured using a ruler, and the mean diameter was estimated using the scale on the SEM. An Electronscan ESEM 2020 (Philips Electron Optics, Eindhoven, The Netherlands) scanning electron microscope operating at 5 kV was used for these measurements.

2.2.3. Drug entrapment efficiency

The amount of psoralen A entrapped within the NP was determined spectrophotometrically (Shimadzu UV-250 1 PC) by a direct and an indirect method. The direct method involved dissolving the NP in methylene chloride and assessing drug