Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 43–53
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

In vivo photodynamic activity of photosensitizer-loaded nanoparticles: Formulation properties, administration parameters and biological issues involved in PDT outcome

Angelica Vargas [a], Michael Eid [b], Mohammed Fanchaouy [c], Robert Gurny [a], Florence Delie [a,*]

[a] *Department of Pharmaceutics and Biopharmaceutics, University of Geneva, University of Lausanne, Switzerland*
[b] *Department of Educational Sciences and Psychology, Free University of Berlin, Germany*
[c] *Department of Zoology and Animal Biology, University of Geneva, Switzerland*

Received 5 May 2007; accepted in revised form 26 September 2007
Available online 12 October 2007

Abstract

Encapsulation of hydrophobic photosensitizers (PS) into polymeric nanoparticles (NP) has proven to be an effective alternative to organic solvents for their formulation. As NP size controls NP passage through endothelial barriers, it is a key parameter for achieving passive targeting of cancer tissues and choroidal neovascularization, secondary to age-related macular degeneration, the main applications of photodynamic therapy. In the present study, a hydrophobic PS, the *meso*-tetra(*p*-hydroxyphenyl)porphyrin, was encapsulated into biodegradable NP made of poly(D,L-lactide-co-glycolide) 50:50 via an emulsification-diffusion technique. NP batches having mean diameters of 117, 285, and 593 nm were obtained with narrow size distribution. Using the chorioallantoic membrane (CAM) of the developing chick embryo, it was demonstrated that the increase in the NP size decreased photodynamic activity *in vivo*. The activity of PS-loaded NP was not influenced by the volume of injection and was kept intact at least 6 h after NP reconstitution. Investigation of NP circulation after IV administration by fluorescence measurements revealed that 117 nm NP reached T_{max} earlier than larger NP. Confocal imaging of CAM vessels demonstrated PS uptake by endothelial cells after NP administration. It was concluded that NP size controls the photodynamic activity of the encapsulated PS.

Keywords: Photodynamic therapy; Polymeric nanoparticles; Nanoparticle size; Chick chorioallantoic membrane (CAM) model; Photosensitizer; *In vivo*; Vascular occlusion; Endothelial cells; Age-related macular degeneration; Cancer

1. Introduction

Photodynamic therapy (PDT) is an innovative alternative to conventional therapies against cancer [1], and the treatment of choroidal neovascularization (CNV), secondary to age-related macular degeneration (AMD), one of the leading causes of blindness in elderly people in developed countries [2]. PDT is based on the systemic or topical administration of photosensitizing drugs, also known as photosensitizers (PS). After biodistribution of the drug, the target tissue is illuminated with light at an appropriate wavelength and dose. Light activates the PS, which, in the presence of molecular oxygen, generates oxidizing species such as singlet oxygen. Such highly cytotoxic species induce cellular damage leading to cell death and alteration of the vasculature in terms of occlusion, stasis and/or increase in vascular permeability [1,3]. The clinical efficacy of PDT is often impeded by the difficulty in administering mostly hydrophobic PS intravenously (IV), and the low selectivity towards target tissues. Biodegradable

* Corresponding author. Department of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30, Quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland. Tel.: +41 22 379 65 73; fax: +41 22 379 65 67.
E-mail address: Florence.delie@pharm.unige.ch (F. Delie).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2007.09.021

nanoparticles (NP) have been proposed as a promising approach to overcome these problems [4].

In vivo studies, recently performed in our group on the chick embryo, demonstrated that the encapsulation of a hydrophobic derivative of a porphyrin into polymeric biodegradable NP allowed the IV administration of the PS [5]. Furthermore, an enhancement of the photodynamic activity of the porphyrin was observed, in terms of the occlusion of blood vessels in the chick embryo chorioallantoic membrane (CAM).

The size of colloidal drug carriers has been shown to govern their passage from the intra- to the extravascular compartment, namely extravasation [6,7]. Therefore, PS extravasation could be controlled by the size of NP in which the PS is incorporated. This strategy could be useful for achieving passive targeting of PS to either cancer tissues or the pathological choroidal neovasculature in AMD. Since the target tissues are different in cancer and CNV–AMD treatments, the optimal NP size differs between both applications, as explained below.

In the case of cancer, NP extravasation is necessary to enrich tumoral tissues in PS, thus increasing PDT outcome. Tumor tissues are characterized by a phenomenon known as enhanced permeability and retention (EPR) effect induced by leaky tumor vasculature and poor lymphatic drainage [8]. The EPR effect results in the accumulation and retention of macromolecules in the perivascular regions of solid tumors to a greater extent than in normal tissues. NP capable of crossing the fenestrations of tumoral capillaries, but large enough to be retained within the interstitial space, are a promising strategy for cancer treatment [4,9].

On the contrary, in CNV–AMD treatment by PDT, NP must be confined within the pathological neovasculature to selectively occlude the neovessels without harming the neighboring tissues, such as the retinal pigmented epithelia or the photoreceptors, failing which, further vision loss can occur. Since choroidal neovasculature exhibits hyper-permeability [10], medium- or large-sized NP that stay inside the choroidal neovasculature would be useful to protect healthy surrounding tissues against phototoxicity.

The objective of this study was to optimize NP with respect to the above-mentioned pathologies. NP of different sizes incorporating a hydrophobic PS were developed, and their *in vivo* activity, in terms of light-induced vascular occlusion, was assessed in the CAM model. The CAM model offers several advantages for the *in vivo* evaluation of PS [11,12]. CAM has a well vascularized membrane, which is easily accessible and easy to handle for PS administration, light irradiation, fluorescence analysis of administered PS, and optical examination of PDT-induced vascular damage [11,12]. Apart from the NP size, some important parameters for potential use in clinics, such as the volume of solvent used for administering a given dose of PS and the effect of NP resuspension after freeze-drying, were evaluated. Furthermore, biological issues related to the NP size, such as the residence time of the NP within the vascular compartment and the uptake of NP by vascular endothelial cells, were investigated.

2. Materials and methods

2.1. Chemicals

Poly(D,L-lactide-co-glycolide) (PLGA) with a copolymer ratio of 50:50 and molecular weight of 12 kDa (Resomer® RG502) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Poly(vinyl alcohol) (PVAL) 87.7% hydrolyzed, with a molecular weight of 26 kDa (Mowiol® 4–88), was obtained from Hoechst (Frankfurt/Main, Germany). Polyethyleneglycol 400 Ph. Eur. (PEG 400) was provided by Merck (Schuchardt, Germany). Concanavalin A labelled with fluorescein isothiocyanate (FITC-Con A), meso-tetra(*p*-hydroxyphenyl)porphyrin (which will be referred to as *m*-THPP), NaCl, phosphate-buffered saline (PBS), and D(+)-trehalose dihydrate were provided by Sigma–Aldrich (Steinheim, Germany). Benzyl alcohol, ethanol 99.8%, propylene carbonate and rhodamine 101 were obtained from Fluka (Buchs, Switzerland), as well as the chemicals used to prepare HEPES-buffered saline: dextrose, HEPES, sodium chloride, sodium phosphate dibasic dihydrate and potassium chloride. All chemicals were of analytical grade and were used without further purification.

2.2. Nanoparticle preparation

PLGA NP of three different sizes loaded with *m*-THPP were prepared using the emulsification-diffusion technique as previously described [5,13]. The experimental conditions for obtaining NP of around 100, 300 and 600 nm were determined after preliminary experimentation and are summarized in Table 1. In a typical procedure, PLGA and *m*-THPP were dissolved in a mixture of benzyl alcohol and propylene carbonate. Three grams of this organic phase was added to 4 g of an aqueous phase containing PVAL and mechanically stirred to form an oil-in-water nanoemulsion. After 15 min of stirring, 500 ml of distilled water was added to the emulsion to induce the formation of the NP after complete diffusion of the organic solvents into the aqueous external phase. Mechanical stirring (2000 rpm) was maintained for 10 min. NP were purified by cross-flow filtration as described previously [13]. Purified NP were freeze-dried in the presence of trehalose (trehalose/NP mass ratio 2:1) with a freeze-dryer Lyolab C II (LSL Secfroid, Aclens, Switzerland).

2.3. Nanoparticle characterization

The mean diameter and the polydispersity index of the freeze-dried NP were determined by photon correlation spectroscopy, and the zeta potential of NP in 10 mM NaCl was measured using the technique of electrophoretic laser Doppler anemometry (Zetasizer® 5000, Malvern,