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Encapsulation of porphyrins and chlorins in biodegradable nanoparticles: The effect of dye lipophilicity on the extravasation and the photothrombic activity. A comparative study

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Abstract

In the present work, we performed a preclinical inter-comparison study using several photosensitizers with the goal of optimizing photodynamic therapy (PDT) for the treatment of choroidal neovascularization (CNV) associated with age-related macular degeneration. The tested molecules were the porphyrins *meso*-tetraphenylporphyrin (TPP) and *meso*-tetra-(4-carboxyphenyl)-porphyrin (TCPP), and the chlorins pheophorbide-a (Pheo-a) and chlorin e_6 (Ce_6). Each of these molecules was entrapped in biodegradable nanoparticles (NP) based on poly(D,L-lactic acid). The influence of the degree of lipophilicity on the incorporation efficiency of the drug in the NPs, and on the dye leakage from blood vessels as well as on the photothrombic efficiency was investigated using the chick chorioallantoic membrane (CAM) as in vivo model. NP characterization showed that the dye was more effectively entrapped in the polymeric matrix when its degree of lipophilicity increased. While less lipophilic compounds (TCPP, Ce_6) extravasate rather easily, the more lipophilic dyes (TPP, Pheo-a) tend to remain inside the blood vessels. After injection of a drug dose of 1 mg/kg body weight and a drug-light application interval of 1 min, irradiation with light doses ranging from 5 to 20 J/cm^2 led to the highest photothrombic efficiency when using the NPs loaded with the most lipophilic molecule (TPP). The latter induced vascular damage, which was significantly higher than that observed with the other molecules tested. Thus, in addition to minimal leakage from blood vessels, the TPP in NP formulation exhibited photothrombic efficiency similar to Visudyne® which was also tested in the CAM model.

Keywords: Photodynamic therapy; Nanoparticles; Photosensitizer; Lipophilicity; Extravasation; Chick embryo; Porphyrins; Chlorins; Verteporfin; CAM model

1. Introduction

The wet form of age-related macular degeneration (AMD), characterized by choroidal neovascularization (CNV), is the main reason for most of the severe, irreversible central vision loss among people in the western world over 50 years of age [1]. Photodynamic therapy (PDT) with liposomal verteporfin or Visudyne® [2,3], the first photodynamic drug to receive regulatory approval for use in ophthalmology, causes a photothrombic effect on CNV [4,5]. In this treatment, PDT aims at closing CNV without affecting neighboring healthy tissue. Since photosensitizers (PSs) at the applied concentrations are essentially non-toxic in light free conditions, a light beam, at the appropriate wavelength, focused onto the area containing the CNV provides a

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simple approach for achieving at least partially selective closure of the CNV. Verteporfin therapy has been shown to cause minimal damage to most normal intraocular tissues with the exception of some closure of normal choriocapillaries and may also cause some damage to the retinal pigment epithelium (RPE) and outer retina [2,6]. As well as some closure of choriocapillaries, the damage of RPE and outer retinal constituents is probably due to the rapid early localization of verteporfin in these tissues following leakage from CNV and/or choriocapillaries. One week after PDT with verteporfin, one observes a significant decrease in perfusion of CNV, but some degree of reperfusion and re-growth of neovascularization commence shortly afterwards, necessitating re-treatment strategies [2,3,7]. During the search to improve the efficacy and safety of PDT of CNV, an effort is being made to design new molecules and improve pharmaceutical formulations [8]. Among the drug delivery systems investigated, colloidal carriers such as nanoparticles (NPs) with their high photosensitizer (PS) loading and their capability to accommodate PSs with widely varying photochemical properties, have come into focus as an alternative delivery system. The incorporation of PS in NPs has been shown in some cases to reduce toxicity, provide solubility in plasma [9], enhance therapeutic activity [10,11], prolong the delivery and, in some cases, provide targeting to specific tissues [12]. In a recent study [13], we have also demonstrated another advantage of the NP carrier that substantially reduces leakage of *meso*-tetra-(hydroxyphenyl)-porphyrin from chick chorioallantoic membrane vasculature besides enhancing the dye's photothrombic activity. This property may well be useful in the photodynamic treatment of CNV in the human eye where one does not wish to damage neighboring compartments like the RPE cells or the photoreceptors. Several parameters including dye physico-chemical properties like lipophilic/hydrophilic balance can strongly influence both the PS delivery with NP formulations and the PDT efficiency of the entrapped drug.

Hence, the goal of the present study was to incorporate PSs with varying hydrophilic/lipophilic balance into biodegradable NPs, in order to assess the impact of the lipophilicity on the extravasation and the photodynamic properties of these PSs. The PSs we selected include both porphyrins (*meso*-tetraphenylporphyrin (TPP) and *meso*-tetra-(carboxyphenyl)porphyrin (TCPP)) and chlorins (chlorin e_6 (Ce_6) and pheophorbide-a (Pheo-a)). Poly(D,L-lactide) with free carboxylic endgroups was used as a biocompatible and biodegradable polymer. The choice of this polymer was guided primarily because of the possibility to subsequently bind specific proteins by a covalent linkage at the surface of the NPs for enhanced targeting [14]. The NP formulations were preclinically evaluated using the chorioallantoic membrane (CAM) of the chicken embryo as in vivo model. Finally, the photothrombic activity of the most promising NP formulation developed in this study was compared with that observed with Visudyne® (i.e., BPD-MA delivered in liposomes) under essentially identical conditions.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (Resomer® R202H) with a molecular weight of approximately 12,000 Da was obtained from Boehringer Ingelheim (Ingelheim, Germany). Chlorin e_6 (Ce_6), pheophorbide-a (Pheo-a) and *meso*-tetra-(*p*-carboxyphenyl)-porphyrin (TCPP) were obtained from Porphyrin Products (Frontier Scientific, Logan, USA). *meso*-Tetraphenylporphyrin (TPP) was provided by Aldrich (Steinheim, Germany). Poly(vinyl alcohol) (PVAL) 87.7% hydrolyzed with a molecular weight of 26,000 Da (Mowiol® 4-88) (Omya AG, Oftringen, Switzerland) and magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) (Fluka Biochemika, Buchs, Switzerland) were selected as stabilizing colloid and salting-out agent, respectively. Tetrahydrofuran (THF) (Merck, Darmstadt, Germany) and benzyl alcohol (Fluka Biochemika, Buchs, Switzerland) were used as organic solvents for the NP preparation. Phosphate-buffered saline (PBS) and dimethylsulfoxide (DMSO) were provided by Life-Technologies (Basel, Switzerland) and Sigma–Aldrich (Steinheim, Germany), respectively. D(+)-Trehalose dihydrate was purchased from Sigma Chemical Co. (St.Louis, MO, USA). Sulforhodamine 101 was purchased from Fluka Biochemika (Buchs, Switzerland). Visudyne® was obtained from Novartis Ophthalmics (Novartis Pharma Inc., Hettlingen, Switzerland).

All other chemicals were of analytical grade and were used without further purification.

2.2. Nanoparticle preparation

The NPs loaded with chlorin or porphyrin derivatives were prepared using emulsification–diffusion [15] and salting-out techniques [16], respectively, using THF and benzyl alcohol, choices dictated by the solubility of the dye. For the salting-out procedure, magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was added to the aqueous solution in order to induce the separation of THF, a water-miscible solvent, from the aqueous solution. A sufficient amount of distilled water was then added to the resulting emulsion to allow essentially complete diffusion of the solvent into the aqueous phase, which then leads to the precipitation of the polymer in the form of NPs. For the emulsification procedure, the organic phase (5 g), composed of polymer (190 mg)