Effect of the lipophilicity on the photothrombic activity of biodegradable nanoparticles loaded with porphyrin derivatives: a comparative study

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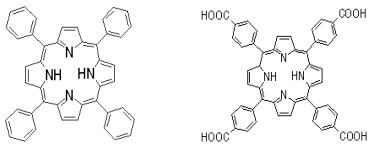
1. Introduction

Photodynamic therapy (PDT) is the most promising therapy currently used for the treatment of choroidal neovascularization due to age-related macular degeneration [1]. To be useful as a therapeutic regimen, some selectivity between the target tissue and the neighbouring healthy structures must be achieved. This could be obtained by choosing a suitable delivery system for the photosensitizer (PS). Hence, the main goal of the present study was to develop a polymeric carrier system such as nanoparticles (NP) for PS intended to be intravenously administrated, capable of increasing the therapeutic index of the PS and devoid of adverse effects. It is known that lipophilicity is one of keys affecting the photodynamic activity of the PS. To assess the effect of this parameter on the properties of the NP in terms of drug efficiency entrapment and photothrombic activity, two porphyrin derivatives with different lipophilic character were selected as molecule models.

2. Materials and Methods

2.1 Photosensitizers

meso-(tetraphenyl)porphyrin (TPP) and meso-tetra(carboxypheny)lporphyrin (TCPP) were obtained from Porphyrin Products (Frontier Scientific, Logan Utah, USA). The chemical structure of the PS is reported in Fig 1. For the control formulation, a solution of each PS (1 mg/ml) was freshly prepared by dissolving in dimethyl sulfoxide (Sigma-Aldrich, Steinheim, Germany).



TPP **TCPP**

Fig 1: chemical structure of the photosensitizers

2.2 Octanol-water partition coefficients

Partition coefficien between 1-octanol and PBS of each photosensitizer was determined according to the shake-flask method described earlier [2]. Briefly, 30 mg of the PS were dissolved in the organic phase (octanol, presaturated with water) and were shaken with PBS (pH 7.4; 20 mM) for three days at room temperature. After separation, drug concentration in both organic and aqueous phases was spectrophotometrically assayed at 416 nm. The octanol-water partition coefficient expressed as Log $P_{o/w}$ is defined as:

$$\text{Log } P_{o/w} = \text{Log } (C_o/C_w)$$

Where Co is the concentration of photosensitizing agent in the octanol phase and Cw its concentration in the aqueous phase when the system is at equilibrium.

2.3 Nanoparticle preparation and characterization

Poly(D,L-lactid acid) with molecular weight of 12 000 provided by Boehringer Ingelheim (Ingelheim, Germany) was used as biodegradable polymer. The NP were prepared using the salting-out technique previously described [3]. The particle suspensions were purified by cross-flow filtration and freeze-dried in the presence of trehalose, (trehalose:nanoparticles mass ratio of 2:1). The particle mean size was assessed by photon correlation spectroscopy using a Zetasizer® 5000 (Malvern, Worcesterhire, England). The drug content and entrapment efficiency were determined spectrophotometrically at 416 nm.

2.4 CAM preparation

For PDT studies, fertilized chicken eggs were disinfected, and transferred into a hatching incubator set at 37°C and 60 % humidity and equipped with an automatic rotator (SARL SAVIMAT, Chauffry, France). On the third day of embryo development (EDD), an approximately 3 mm diameter hole was bored into the eggshell at the narrow apex and covered with a cling foil. Egg incubation was then continued in a static position. On EDD13, the holes were extended to a diameter of about 3 cm and the embryos were placed under an epi-fluorescence microscope (Nikon Eclipse E600 FN, Japan).

2.5 Phototoxicity assays

Prior to injection, an autofluorescence image of CAM (EDD13) surface was recorded. Subsequently, injection of the dye (1 or 0.5 mg/kg body weight (b.w.)) was performed in situ under the microscope and its circulation was observed by fluorescence microscopy. Following the homogenous distribution of the photosensitizer in the blood circulation, PDT was performed using a filtered Hg-arc lamp at 420 $\pm$ 20 nm at a fluence rate of 141 mW/cm^2. Light doses ranging from 5 to 20 J/cm^2 were applied to the CAM. Then, the eggs were covered with a cling foil and returned to the incubator for 24 hours. Then, vascular occlusion was observed by means of fluorescence angiography, performed by injection of 10 μl of sulforhodamine 101 (5 mg/ml). Fluorescence angiographies pre- and post- PDT were compared and scored according to the arbitrary score of Table 1.

Table 1: Damage criteria for PDT in the CAM vessels.

Damage scale	Criterion
0	- No damage.
1	- Partial closure of capillaries (∅ < 10 μm).
2	- Closure of capillaries, partial closure of blood vessels (∅ < 30 μm) and size reduction of bigger blood vessels.
3	- Closure of vessels (∅ < 30 μm) and partial closure of bigger vessels.
4	- Total closure of vessels (∅ < 70 μm) and partial closure of bigger vessels.
5	- Total occlusion of the irradiated area.

3. Results and discussions

3.1 Nanoparticle characterization

Irrespective the PS used, NP with a mean size around 200 nm were reproducibly prepared using the same process parameters (Table 2). Nevertheless, the NP loaded with the more hydrophobic dye (Log *P* = 4.8) has far greater loading efficiency and amount compared to those loaded with TCPP (Log *P* = 2.3). This is because the more hydrophobic dye had limited water solubility, suppressing the tendency of the PS to escape out of NP into aqueous medium during the purification process. On the other hand, the NP formulated with the less hydrophobic PS exhibited lower loading efficiency and amount due to their greater aqueous solubility (Table 2).

Table 2: Characterization of PS-loaded NP freeze-dried in the presence of trehalose (mean ± SD, n= 3).

PS	Log $P_{o/w}$	Mean NP size (nm)	Drug loading (%)	Entrapment efficiency (%)
TPP	4.8	210 ± 11	4.57 ± 0.06	87.2 ± 1.0
TCPP	2.3	194 ± 12	1.07± 0.08	29.9 ± 0.5

3.2 PDT assays

Comparing both free PS solutions (Fig.3), it can be observed that free TCPP exhibited the highest vascular damage and this, irrespective the light dose applied. This could be explained by the fact that due to its own lipophilic character, free TPP tends to aggregate in aqueous media as a result of the propensity of the hydrophobic skeleton to avoid contact with water molecules. This aggregate state can hinder the efficiency of the PS photoactivity since it can limit the TPP capacity to absorb light [4].

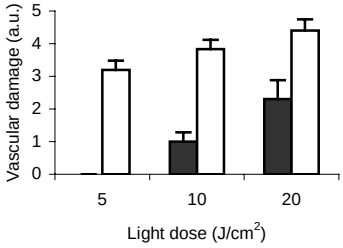


Fig. 3: Vascular damage of free TPP (■) or TCPP (□). The injected drug dose was **1 mg/kg b.w**.

On the other hand, encapsulated into NP, TPP was proved to be more efficient than NP-loaded TCPP (Fig 4) at the same drug dose. Indeed, irrespective the light dose applied, total occlusion of the irradiated area was observed with TPP-loaded NP.

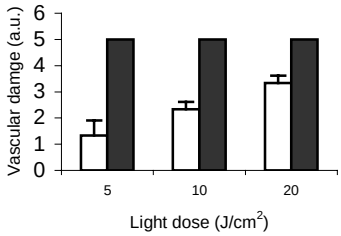


Fig. 4.: Vascular damage obtained with NP loaded with TPP (■) or TCPP (□). The injected drug dose was **1 mg/kg b.w.**

In addition, lowering the drug dose at 0.5 mg/kg b.w., encapsulated TPP exhibited similar extent of vascular damage than TCPP-loaded NP at dose of 1 mg/kg b.w. (Fig. 5). The higher photothrombic efficiency of TPP-loaded NP compared to TCPP formulation could be explained by different hypothesis. First, the nanoencapsulation probably favour the monomeric state of TPP, the main highly photoactive form. Depending on the degree of dye lipophilicity, the NP could be accumulated into endothelial cells via different mechanisms and/or rate leading to different intracellular localization.

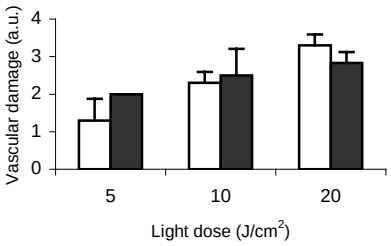


Fig. 5: Photodynamic activity of NP loaded with TPP (■) at drug dose of **0.5 mg/kg** b.w. compared to those loaded with TCPP (□) at drug dose of **1 mg/kg** b.w.

4. Conclusions

In this study, we have demonstrated that the incorporation of PS into NP was strongly influence by the hydrophobic/hydrophilic character of the molecules. This parameter was proved to be one of main factors governing the NP properties in terms of phototherapeutic activity. Indeed, NP formulated with the more hydrophobic PS exhibited the highest entrapment efficiency and extent of vascular damage.

5. Reference

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2) Uehlinger et al., *J. Photochem. Photobiol. B: Biology,* 54 (2000) 72-80.
3) Konan et al*, Int. J. Pharm*. 233 (2002) 239-252.
4) Csik et al., *J. Photochem. Photobiol. B: Biology,* 44 (1998) 216-224.