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Research paper

Plasma distribution of tetraphenylporphyrin derivatives relevant for Photodynamic Therapy: Importance and limits of hydrophobicity

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ABSTRACT

In the course of a Photodynamic Therapy (PDT) protocol, disaggregation of the sensitizer upon binding to plasma proteins and lipoproteins is one of the first steps following intravenous administration. This step governs its subsequent biodistribution and has even been evoked as possibly orientating mechanism of tumor destruction. It is currently admitted as being mainly dependent on sensitizer's hydrophobicity. In this context, as far as glycoconjugation of meso-tetraphenylporphyrin (TPP) macrocycle, a promising strategy to improve targeting of retinoblastoma cells confers to the sensitizer an amphiphilic character, we have studied the effect of this strategy on binding to plasma proteins and lipoproteins. With the exception of the majoritary protein binding (more than 80%) of more hydrophilic *para*-tetraglycoconjugated derivatives, high density lipoproteins (HDL) appear as main plasma carriers of the other amphiphilic glycoconjugated photosensitizers. This HDL-binding is a combined result of binding affinities (log K_a ranging from 4.90 to 8.77 depending on the carrier and the TPP derivative considered) and relative plasma concentrations of the different carriers. Evaluation of binding affinities shows that if hydrophobicity can account for LDL- and HDL-affinities, it is not the case for albumin-affinity. Molecular docking simulations show that, if interactions are mainly of hydrophobic nature, polar interactions such as hydrogen bonds are also involved. This combination of interaction modalities should account for the absence of clear relationship between albumin-affinity and hydrophobicity. Taken together, our findings clarify the importance, but also the limits, of hydrophobicity's role in structure–plasma distribution relationship.

1. Introduction

Photodynamic Therapy (PDT) is an emerging technique which combines administration of a drug, called photosensitizer, and exposure of targeted tissue to light of appropriate wavelength. Treatment effect results from the potency of the photosensitizer once activated by light to generate singlet oxygen and radical species responsible for cellular death. PDT has already proven its efficacy in the field of oncology for the treatment of lung, gastrointestinal or cutaneous tumors. It has also been applied to non malignant diseases such as age-related macular degeneration [1]. In that case, transparency of ocular tissues to light makes PDT of particular interest. This property should also be exploited for the treatment of malignant ocular pathologies, such as retinoblastoma, the most frequent intraocular tumor in childhood. Indeed, besides poor efficiency for advanced tumors, currently available conservative treatments expose patients to a risk of

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; HSA, human serum albumin (essentially fatty acid free); HSAlip, human serum albumin (not fatty acid free); TPP, 5,10,15,20-tetraphenylporphyrin, *meso*-tetraphenylporphyrin; MCR-ALS, multivariate curve resolution – alternating least squares; TPP(*p*OH)$_4$, 5,10,15,20-tetra-(*para*-hydroxyphenyl)porphyrin; TPP(*p*OβGalOH)$_3$, 5,10,15-tri(*para*-O-β-D-galactosyloxyphenyl)-20-phenylporphyrin; TPP(*p*OβGalOH)$_4$, 5,10,15,20-tetra-(*para*-O-β-D-galactosyloxyphenyl)porphyrin; TPP(*p*OβGluOH)$_4$, 5,10,15,20-tetra-(*para*-O-β-D-glucopyranosyloxyphenyl)porphyrin; TPP(*p*ODEGOαManOH)$_3$, 5,10,15-tri{*para*-O-[(2-(2-O-α-D-mannosyloxy)-ethoxy)-ethoxy]-phenyl}-20-phenylporphyrin.

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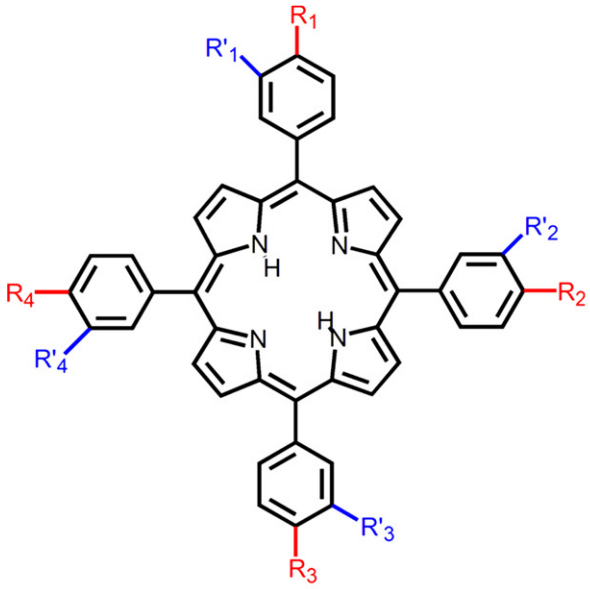
developing secondary tumors [2]. PDT appears as promising, combining a physical selectivity (tissular volume illuminated) and a chemical one (tissular volume containing the photosensitizer). When applied to retinoblastoma tumors, photosensitizers developed for other pathologies have shown poor efficiencies and selectivities, leading to side-effects such as long lasting photosensitization of normal tissues. Design of new photosensitizers adapted to retinoblastoma appears necessary [3].

Our group is involved in the evaluation of glycoconjugation of tetrapyrrolic macrocycles (Fig. 1). This strategy combines targeting of cellular sugar receptors and improvement of photosensitizer solubility. The former promotes selective destruction of malignant cells, the latter favors rapid elimination from healthy tissues. In vitro photocytotoxicity and in vivo pharmacokinetics studies have confirmed the potential interest of this approach [4,5]. Efficacy of a glycoconjugated TPP, TPP(*p*ODEGOαManOH)$_3$, has been attested in vivo, especially with a particular administration protocol (double drug dose with a 3 h interval), which combines targeting of cancer cells and of blood vessels. Indeed, at the time of illumination, drug administered 10 min before is still present in the vicinity of blood vessels, whereas drug administered 3 h before has reached tumor cells [6]. Destruction of blood vessels indirectly kills tumor tissue, through deprivation of oxygen and nutriments [7].

Photo-induced destruction of blood vessels is of particular interest in the case of an application of PDT to retinoblastoma as far as this tumor is considered as extremely sensitive to vascular insufficiency [8]. Besides being a candidate target to PDT treatment, tumor vasculature has also been involved in sensitizer sequestration in tumor tissue [9]. Indeed, tumor angiogenesis leads to the formation of permeable neo-vessels, a specificity which has



***meta*-substituted compounds**				
$R_1 = R_2 = R_3 = R_4 = -H$	R'_1	R'_2	R'_3	R'_4
TPP(*m*OH)$_3$	-OH	-OH	-OH	-H
TPP(*m*OH)$_4$	-OH	-OH	-OH	-OH
TPP(*m*OβGluOH)$_3$	-OβGluOH	-OβGluOH	-OβGluOH	-H
TPP(*m*OβGluOH)$_4$	-OβGluOH	-OβGluOH	-OβGluOH	-OβGluOH
***para*-substituted compounds**				
$R'_1 = R'_2 = R'_3 = R'_4 = -H$	R_1	R_2	R_3	R_4
TPP(*p*OH)$_3$	-OH	-OH	-OH	-H
TPP(*p*OH)$_4$	-OH	-OH	-OH	-OH
TPP(*p*OβGalOH)$_3$	-OβGalOH	-OβGalOH	-OβGalOH	-H
TPP(*p*OβGalOH)$_4$	-OβGalOH	-OβGalOH	-OβGalOH	-OβGalOH
TPP(*p*OβGluOH)$_4$	-OβGluOH	-OβGluOH	-OβGluOH	-OβGluOH
TPP(*p*ODEGOαManOH)$_3$	-ODEGOαManOH	-ODEGOαManOH	-ODEGOαManOH	-H

Fig. 1. Structure of *meso*-tetraphenylporphyrin derivatives. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)