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Glycodendrimeric phenylporphyrins as new candidates for retinoblastoma PDT: Blood carriers and photodynamic activity in cells

Ze-Jian Wang [a], Benoît Chauvin [a,*], Philippe Maillard [b,c], Fabien Hammerer [b,c], Danièle Carez [b,d], Alain Croisy [b,d], Catherine Sandré [e], Sylvie Chollet-Martin [e], Patrice Prognon [a], Jean-Louis Paul [f,g], Jocelyne Blais [a], Athena Kasselouri [a,*]

[a] EA 4041, Laboratoire de Chimie Analytique, IFR 141, Univ. Paris-Sud, 5 rue J.-B. Clément, F-92296 Châtenay-Malabry, France
[b] Institut Curie, Centre de Recherche, Centre Universitaire, Univ. Paris-Sud, F-91405 Orsay, France
[c] CNRS, UMR 176, Centre Universitaire, Univ. Paris-Sud, F-91405 Orsay, France
[d] INSERL, U 759, Centre Universitaire, Univ. Paris-Sud, F-91405 Orsay, France
[e] Laboratoire d'Immunologie, Inserm UMR 996, IFR 141, Univ. Paris-Sud, 5 rue J.-B. Clément, F-92296 Châtenay-Malabry, France
[f] AP-HP, Hôpital Européen Georges Pompidou, Service de Biochimie, 20 Rue Leblanc, F-75015 Paris, France
[g] EA 4529, Laboratoire de Biochimie appliquée, Univ. Paris-Sud, 5 rue J.-B. Clément, F-92296 Châtenay-Malabry, France

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ABSTRACT

Photodynamic therapy (PDT) has recently been proposed as a possible indication in the conservative treatment of hereditary retinoblastoma. In order to create photosensitizers with enhanced targeting ability toward retinoblastoma cells, *meso*-tetraphenylporphyrins bearing one glycodendrimeric moiety have been synthesized. The binding properties to plasma proteins and photodynamic activity of two monodendrimeric porphyrins bearing three mannose units via monoethylene glycol (**1**) or diethylene glycol (**2**) linkers have been compared to that of the non-dendrimeric tri-substituted derivative [TPP(*p*-Deg-O-α-ManOH)$_3$]. The dendrimeric structure was found to highly increase the binding affinity to plasma proteins and to modify to some extent plasma distribution. HDL and to a lesser extent LDL have been shown to be the main carriers of dendrimeric and non-dendrimeric compounds. The phototoxicity observed for the two glycodendrimers (**1**) and (**2**) (LD_{50} = 0.5 μM) in Y79 cells is of the same order of magnitude that for TPP(*p*-Deg-O-α-ManOH)$_3$ (LD_{50} = 0.7 μM), with a similar cellular uptake level for (**1**) and a lower for (**2**). A serum content increase from 2% to 20% (v/v) in the incubation medium was found to inhibit both cellular uptake and photoactivity of dendrimeric derivatives, whereas those of TPP(*p*-Deg-O-α-ManOH)$_3$ remained little affected. Specificities of glycodendrimeric porphyrins, combining a lower cellular uptake together with a higher affinity toward plasma proteins, make these derivatives possible candidates for a vascular targeting PDT.

1. Introduction

Retinoblastoma is the most common malignant intraocular tumor in children. Among the conservative treatments now available, the chemotherapy with carboplatin appears as effective in the advanced forms of retinoblastoma but presents short and long-term side effects [1]. The development of an alternative treatment involving photodynamic therapy (PDT) is thus of particular interest [2]. PDT is based on the administration of a non-mutagenic photosensitizer (PS) (usually a tetrapyrrolic macrocycle) followed by the exposure of the pathological area to visible light [3]. Until now, due to the poor efficiency and side effects of the PS used, few attempts have been made to use PDT in the treatment of retinoblastoma [2]. In this context, many efforts have been focused in our group on the development of new photosensitizers and glycoconjugated *meso*-tetraphenylporphyrins have been synthesized [4]. In addition to an improvement of the water solubility of the PS, the glycoconjugation can be an effective strategy for targeting lectin-type receptors which have been shown to be overexpressed onto tumor cell membranes. Griegel et al. established that human retinoblastoma cells overexpressed mannose and galactose receptors [5]. Recently, Laville et al. established that TPP(*p*-Deg-O-α-ManOH)$_3$ a tri-mannosylated derivative bearing three mannose residues via three diethylene glycol (Deg) spacers (Fig. 1), exhibited some specific affinity toward Y79 cell membranes and high photobiological activity [4].

It has been shown that multivalent carbohydrate interactions with more than one receptor binding site could result in a better cellular recognition [6]. In order to allow such a "cluster effect" in the PS interactions with cell membrane, three mannose residues

* Corresponding authors. Tel.: +33 1 46835849; fax: +33 1 46835389 (A. Kasselouri), tel.: +33 1 46835476; fax: +33 1 46835389 (B. Chauvin).
E-mail addresses: benoit.chauvin@u-psud.fr (B. Chauvin), athena.kasselouri@u-psud.fr (A. Kasselouri).

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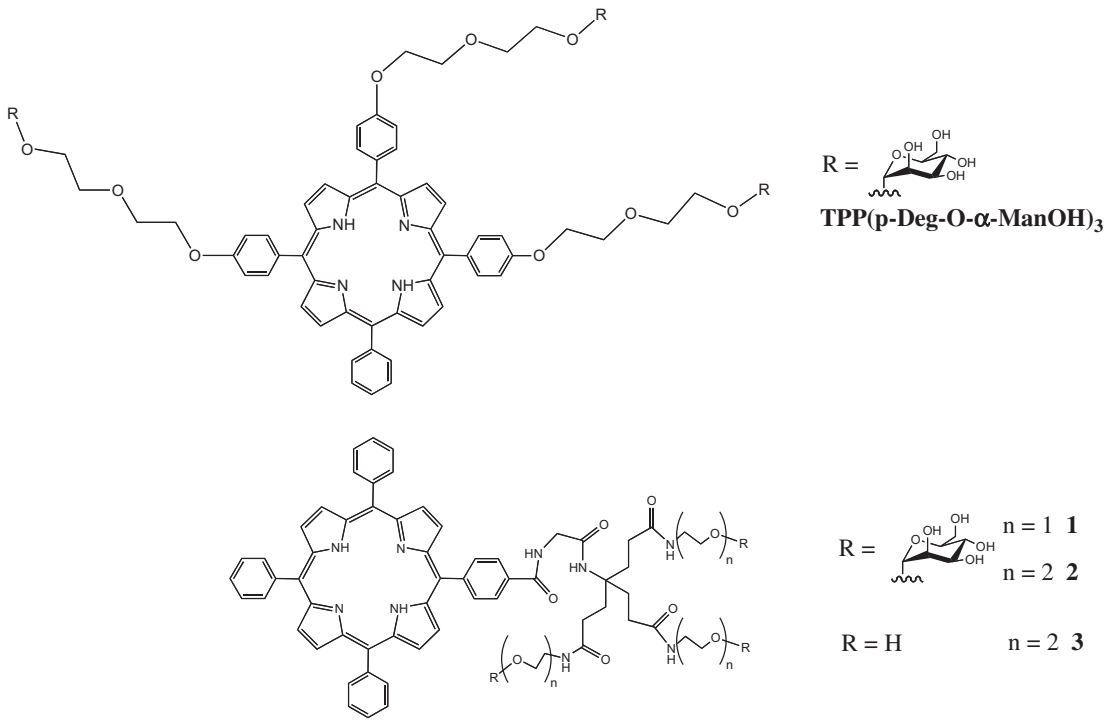

Fig. 1. Chemical structure of mannosylated porphyrins TPP(*p*-Deg-O-α-ManOH)$_3$, (**1**), (**2**), and diethyleneglycol dendrimeric porphyrin (**3**).

have been linked, via ethylene glycol spacers, to the same phenyl of the tetraphenylporphyrin core, conferring to the molecule a dendrimeric structure [7,8] (Fig. 1). Such a structure which strongly affects the lipophilicity and the steric hindrance of the glycoconjugated porphyrin could result in modifications in the binding to plasma proteins and *in vitro* biological and photobiological characteristics.

The distribution of porphyrin derivatives between plasma proteins is an important parameter to consider for a potential *in vivo* use in as far as low density lipoprotein (LDL) receptors could also be overexpressed in cancer cells [9]. Indeed, the photosensitizer-LDL association has been claimed by several authors to favor the internalization of the drug by cancer cells [10]. In contrast, photosensitizers bound to albumin would preferentially accumulate in the vascular stroma of the tumor tissue. Two different photodynamic mechanisms would thus have to be considered: a direct cellular toxic effect in the former case and a vascular damage in the other one [11]. Recently, the non-dendrimeric TPP(*p*-Deg-O-α-ManOH)$_3$ was used to treat human retinoblastoma xenographs in mice. The ^{1}H- and ^{23}Na MRI follow up of the PDT effect clearly established that the treatment was effective when both blood vessels and cancer cells were targeted [12].

In the present work, we have investigated the modifications induced by a dendrimeric structure on photosensitizer plasma distribution, cellular internalization and photobiological activity in human retinoblastoma cells. Two glycodendrimeric *meso*-tetraphenylporphyrins, (**1**) and (**2**), bearing three mannose residues via only one dendrimeric spacer were compared to the non-dendrimeric tri-substituted glycoconjugated tetraphenylporphyrin, TPP(*p*-Deg-O-α-ManOH)$_3$ (Fig. 1). As a control, a non-glycoconjugated dendrimer (**3**) analog of (**2**) was also studied.

2. Materials and methods

All solvents used were reagent grade. The following reagents have been abbreviated: 1-hydroxybenzotriazole hydrate (HOBt), dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (EDC), triethyl amine (Et_3N), dimethylesulfoxide (DMSO), fetal bovine serum (FBS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffer saline (PBS), and Dulbecco's modified Eagle's medium (DMEM). Human serum albumin (HSA) (>99%) was purchased from Sigma–Aldrich.

Dendrimeric porphyrins **1**–**3** were prepared and characterized as previously reported [8]. Briefly, dendrimeric moieties (obtained from di-t-butyl 4-[2-(t-butoxycarbonyl)ethyl]-4-amino-heptanedicarboxylate by a protection–deprotection sequence and N-amidation by glycosylated O-alkyl amines) were linked with 5-*para*-benzoic acid-10,15,20-triphenyl porphyrin using a mixture of HOBt, EDC and Et_3N to give protected glycodendrimeric porphyrins which then were quantitatively O-deacetylated under Zemplén's conditions to afford glycodendrimeric porphyrins **1** and **2**.

TPP(*p*-Deg-O-α-ManOH)$_3$ was prepared from 5,10,15-(tri-*para*-phenol)-20-phenyl porphyrin by a modified Willamson's protocol, via reacting with Br–$(CH_2CH_2O)_2$–O–Mannose–OAc in DMF, followed by O-deprotection as described by Laville et al. [4]. The structure of all compounds was checked by mass spectroscopy, using the positive ion (MH^+) MALDI-TOF spectra. Furthermore, all photosensitizers were characterized by ^{1}H and ^{13}C NMR, homonuclear correlation (COSY), and heteronuclear multiple coherence (HMQC) spectra and elementary analysis.

UV–visible spectra were obtained using a CARY 100 Bio UV–visible spectrophotometer (Varian, USA). Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B (Massachusetts, USA) equipped with a red sensitive photomultiplier.

2.1. Physicochemical properties

Fluorescence quantum yields were determined by a comparative method using *meso*-5,10,15,20-tetra-(*meta*-hydroxyphenyl)porphyrin in methanol as standard [13]. The compounds were classified in terms of lipophilicity by means of their apparent retention factor in gradient elution (k_g) [14] obtained on an octadecyl stationary phase. The apparent retention factor has been calculated as $k_g = (t_g - t_0 - t_D)/t_0$ with t_g the retention time of the