Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology B: Biology 80 (2005) 1–7
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Fullerene-pyropheophorbide *a* complexes as sensitizer for photodynamic therapy: Uptake and photo-induced cytotoxicity on Jurkat cells

Fiorenza Rancan [a,b,*], Matthias Helmreich [c], Andreas Mölich [d], Norbert Jux [c], Andreas Hirsch [c], Beate Röder [b], Christian Witt [e], Fritz Böhm [a]

[a] *Humboldt Universität, Universitätsklinikum Charité, Hautklinik, Photobiologisches Labor, Ziegelstr. 5-9, D-10117 Berlin, Germany*
[b] *Humboldt Universität, Institut für Physik, Newtonstr. 15, D-12489 Berlin, Germany*
[c] *Institut für Organische Chemie, Universität Erlangen-Nürnberg, Henkestr. 42, D-91054 Erlangen, Germany*
[d] *Humboldt Universität, Institut für Biologie, Tierphysiologie, Philippstr. 13, D-10115 Berlin, Germany*
[e] *Humboldt Universität, Universitätsklinikum Charité, Abteilung Pneumologie, Ziegelstr. 5-9, D-10117 Berlin, Germany*

Received 17 September 2004; received in revised form 7 January 2005; accepted 23 January 2005
Available online 23 March 2005

Abstract

The main challenge in searching for new photosensitizers is to improve their specificity for target cells to avoid toxicity towards normal cells. New modular drug delivery systems were proposed consisting of a multiplying unit with the property of carrying several drug moieties and an addressing unity with high selectivity for target cells. Following this concept, two new fullerene-bis-pyropheophorbide *a* derivatives were synthesized: a mono-(FP1) and a hexa-adduct (FHP1). The photophysical characterization of the compounds revealed significantly different parameters related to the number of addends at the fullerene core. In this study, the derivatives were tested with regard to their intracellular uptake and photosensitizing activity towards human leukemia T-lymphocytes (Jurkat cells) in comparison with the free sensitizer, pyropheophorbide *a*. The C_{60}-hexa-adduct FHP1 resulted to have a significative phototoxic activity (58% dead cell, after a dose of 400 mJ/cm^2, 688 nm) while the mono-adduct FP1 had a very low phototoxicity and only at higher light doses. The photosensitizing activity of the fullerene hexa-adduct, FHP1, resulted to be lower than that of pyropheophorbide *a*. The lesser intracellular concentration reached by the C_{60}-hexa-adduct FHP1 is probably the reason for its lower phototoxicity with respect to pyropheophorbide *a*.

Keywords: PDT; Pyropheophorbide *a*; Fullerene; Jurkat cells; Carrier system

1. Introduction

Drug carrier systems play a crucial role in the development of new strategies for photodynamic therapy (PDT) [1,2] as well as for all therapies in which a higher selectivity towards the biological target is needed in order to avoid intolerable side effects [3]. Besides their selectivity, such systems offer also the possibility of a controlled drug release [4] or a prolonged drug effect due to protection from degradation enzymes [5]. Recently, new modular drug delivery systems were proposed consisting of an addressing unit, a multiplying unit and the biological active unit [6]. The addressing unit has the function to recognize selectively the desired target, e.g., a monoclonal antibody which recognizes structures peculiar to tumor cells [7]. The multiplying unit has the function of increasing the number of drug molecules that can be addressed to the target via a single addressing unit. Dendrimers are particularly useful as

* Corresponding author. Tel.: +49 30 450 518085; fax: +49 30 450 513941.
E-mail address: fiorenza.rancan@charite.de (F. Rancan).

1011-1344/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2005.01.007

multiplying units because of their well-defined structure and the high number of end groups which enable them to carry several drug molecules [8–10]. Moreover, thanks to their size and physicochemical properties, dendrimers can exhibit selectivity for solid tumors [11]. The accumulation in tumor tissue of high sized molecules is a passive process and is also known as enhanced permeability and retention (EPR) effect [12].

In preparing such modular carrier systems, particular devices have to be adopted to avoid steric interactions between the photosensitizing molecules and a possible inactivation of the addressing unit. Beside the introduction of spacers, the use of C_{60}-fullerene as a starting block is particular useful. Fullerene hexakisadducts have an octahedral addition pattern, which allows the six substituents to occupy spread positions with minimal steric interactions [13]. So-called 5,1-hexa-adducts are well known in fullerene chemistry. In such adducts, one position can be occupied by the addressing unit and the other five positions are suitable for dendrimers bearing drug moieties. Nevertheless, fullerenes ability to scavenge free radicals [14] and its spherical shape which could adapt to active sites of enzymes [15], makes its use in medical research particularly interesting.

It is well known that in fullerene-tetrapyrrole dyads photoinduced electron transfer processes can take place [e.g., 16, 17]. This leads to a reduction of singlet oxygen quantum yield (Φ_Δ) which is an unwanted effect when synthesizing new photosensitizers. Singlet oxygen plays an important role in the tumor killing process. This excited form of molecular oxygen has a high reactivity and once formed it reacts immediately with cellular constituents producing a high level of reactive oxidative species and other radicals which lead to cell death. Therefore a reduction of Φ_Δ caused by other photodynamic processes is, in most of the cases, the reason for a low phototoxic activity.

Another problem of fullerene derivatives is their low solubility in solvents suitable for biological use. To work on these problems, two fullerene derivatives were synthesized, a mono-(FP1) and a hexa-(FHP1) adduct bearing each two pyropheophorbide *a* moieties [18]. The photophysical characterization of these fullerene derivatives revealed significantly different electronic properties which are related to the number of addends [19,20]. A summary of the photophysical parameters is reported in Table 1. In the C_{60} mono-adduct FP1, an efficient photoinduced electron transfer occurs accompanied by a dramatic reduction of singlet oxygen quantum yield, while in the hexa-adduct FHP1 no electron transfer takes place. For this compound, a Φ_Δ comparable to that of the fullerene-free derivative P2 was measured [20]. The Φ_Δ of FP1 was found to be higher in toluene (0.29) than in DMF (0.03) [20]. It could be thought that this is due to aggregation. Anyway, no broadening of the bands was observed in both absorption and fluorescence spectra of the compounds in DMF [19] as well as in toluene. The low Φ_Δ in DMF is due to the depopulation of the first singlet exited state caused by the electron transfer process between photoexited pyropheophorbide and fullerene. This process is extremely favoured in DMF because the charge-separated state as a low energy, lower than that of the triplet state of pyropheophorbide moiety. In toluene, the charge-separated state lays in between the singlet state and the triplet state. Therefore, in toluene a certain number of molecules will be allowed to populate the triplet state by transition from the charge-separated state and consequently the yield of singlet oxygen will be higher. If the π-system of the fullerene, C_{60} is broken up by exaaddition the probability of electron transfer is significantly reduced and FHP1 shows the same behaviour as P2 [19,20]. In addition, the solubility in the polar solvent dimethylformamide (DMF) was higher for the hexa-adduct than for the mono-adduct derivative, thanks to the five diethyl malonate addends in the remaining positions.

Table 1
Photophysical parameters of compounds P1, P2, FP1 and FHP1 dissolved in DMF

	Q (nm)	ε (M^{-1} cm^{-1})	φ	Φ_Δ
P1	667.7	83,378	1	0.50 ± 0.05
P2	667.5	83,200	0.77 ± 0.002	0.43 ± 0.05
FP1	668.5	40,685	0.09 ± 0.002	0.03 ± 0.05
FPH1	667.7	69,112	0.77 ± 0.002	0.43 ± 0.05

The wavelength at the maximum of the last Q-band (Q), the molar extinction coefficient (ε) at the maximum of the last Q band, the fluorescence quantum yields (φ) relative to P1 and the singlet oxygen quantum yields (Φ_Δ) are reported.

In this in vitro study, we studied the intracellular uptake and verified the photosensitizing ability of the two fullerene-sensitizer complexes FP1 and FHP1, comparing them with those of pyropheophorbide *a* and its derivatives.

2. Materials and methods

2.1. Chemicals

N,*N*-Dimethylformamide (DMF), trypan blue (TB) and staurosporine were purchased from Sigma (Germany), 4′,6-diamidino-2-phenylindol dihydrochloride (DAPI) from Roth GmbH (Germany). The caspase-3/7 assay kit (Apo-ONE™ Homogeneous Caspase-3/7 Assay) was purchased by Promega.

2.2. Pyropheophorbide a and fullerene derivatives

In Fig. 1, the chemical structures of pyropheophorbide *a* (Pyro) and the other derivatives are reported. Pyro was obtained by in situ hydrolysis and decarboxylation of pheophorbide *a* according to a literature protocol [21,22]. The syntheses and spectroscopic data are