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Release kinetics of an amphiphilic photosensitizer by block-polymer nanoparticles

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ABSTRACT

Block-polymer nanoparticles are now well-known candidates for the delivery of various non-soluble drugs to cells. The release of drugs from these nanoparticles is a major concern related to their efficiency as nanovectors and is still not completely deciphered. Various processes have been identified, depending of both the nature of the block-polymer and those of the drugs used. We focused our interest on an amphiphilic photosensitizer studied for photodynamic treatments of cancer, Pheophorbide-a (Pheo). We studied the transfer of Pheo from poly(ethyleneglycol-b-ε-caprolactone) nanoparticles (I) to MCF-7 cancer cells and (II) to models of membranes. Altogether, our results suggest that the delivery of the major part of the Pheo by the nanoparticles occurs *via* a direct transfer of Pheo from the nanoparticles to the membrane, by collision. A minor process may involve the internalization of a small amount of the nanoplatforms by the cells. So, this research illustrates the great care necessary to address the question of the choice of such nanocarriers, in relation with the properties – in particular the relative hydrophobicity – of the drugs encapsulated, and gives elements to predict the mechanism and the efficiency of the delivery.

1. Introduction

The therapeutic use of photosensitizing drugs is based on the light-induced generation of reactive oxygen species that damage surrounding biological structures (Spikes, 1982). These therapies are based on the use of photosensitizers, non-toxic in the dark but capable of generating, under light irradiation, active molecular species such as free radicals and singlet oxygen that are toxic for the biological environment. In terms of medical indications, the potential of these techniques is related to the capability of a number of photosensitizer to accumulate selectively in proliferating tissues, and has been improved by the development of laser diodes and optical fibers (Van den Bergh, 1998). The photosensitizers are used in the clinical treatment of several oncologic and ophtalmologic diseases (Ackroyd et al., 2001; Brown et al., 2004; Levy and Obochi, 1996; Miller et al., 1999). The major advantage of these techniques, named photodynamic therapies (PDT) is their dual-selectivity, resulting from both (i) the positive ratio of photosensitizer accumulation between proliferating and normal surrounding tissues and (ii) the possibility to restrict the light irradiation to the diseased area (Dougherty, 1985; Dougherty et al., 1998). However, the particular photophysical properties of the photosensitizers are related to their macrocycles, and one important limitation for their biomedical uses is due to their consequent amphiphilic or hydrophobic nature. Such drugs require delivery systems able to limit self-assembly and aggregation in aqueous physiologic medium. If this problem occurs for all water-insoluble drugs and involves important problems of administration, cellular incorporation and pharmacokinetics, it is of particular importance in the context of photodynamic therapy, based on the specific photophysical properties of the photosensitizers, that are lost upon aggregation of the dye.

Synthetic vectors such as liposomes and polymeric nanoparticles are increasingly developed for drug delivery, with the goal to solve the solubility-related problems previously mentioned, to improve the biocompatibility of drug delivery systems, protect the therapeutic payload from degradation, delay uptake by the reticuloendothelial system (Wattendorf and Merkle, 2008), enhance the crossing of biological barriers, and efficiently transfer the drug to the target. In this context, improvements of PDT strategies have been obtained by using appropriate formulations (Cremaphor) and nanocarriers able to accumulate within tumors trough the enhanced permeation and retention (EPR) effect, such

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as liposomes (Derycke and de Witte, 2004; Kuntsche et al., 2010) or nanoparticles (Couleaud et al., 2010; Li et al., 2007).

Amphiphilic block copolymer nanoparticles are stable frozen micelles with a diameter between 10 and 100 nm (Kazunori et al., 1993). Their structure exhibit an hydrophobic core able to incorporate drugs, surrounded by a hydrophilic corona ensuring the stability of the micelle (Allen et al., 1999a). This enables them to increase the solubility of hydrophobic compounds (Kim et al., 2011; Liu et al., 2004; Stenzel, 2008; Uhrich et al., 1999). Like other synthetic vectors, they can be designed to handle organism barriers and self-defense (Luo et al., 2002; Owens and Peppas, 2006). Consequently, as nano-scaled delivery platforms, they can be utilized to accumulate the drug in cancerous tissues by EPR effect (Konan et al., 2002; Maeda et al., 2000). As promising vectors, many studies question their effects on solubility, pharmacokinetics and the biodistribution of drugs (Albertsson and Varma, 2003; Kim et al., 2010; Wei et al., 2009; Yokoyama, 2010). Nevertheless, one key point of their efficiency is the mechanism of drug delivery to the cells, which is still not well understood for such stable frozen nanoparticles. Various processes have been proposed. For instance, fluorescent poly (ethyleneoxide-b-ε-caprolactone) (PEO–PCL) copolymer nanopaticles have been suggested to enter the cells by endocytosis (Allen et al., 1999b) and have also been reported to distribute in various intracellular organelles—lysosomes, Golgi aparatus, endoplasmic reticulum and mitochondria (Savic et al., 2003). In contrast, similar nanoparticles, made of (polyethylene glycol)-b-poly (D, L-lactide) (PEG–PDLLA), have been reported to be unable to penetrate into the cells but to transfer the entrapped drug through the plasma membrane, leading to the internalization of the drug (Chen et al., 2008). The mechanism of penetration remains then uncertain.

In this paper we decipher the drug delivery mechanism for one promising type of nanoparticles, made of poly(ethylene oxide)-poly(ε-caprolactone) [PEO(5000)–PCL(4000)]. The entrapped drug, pheophorbide-a (Pheo), is a photosensitizer, the presence of which can be reported by the direct fluorescence of the compound. The uptake by MCF-7 cancer cells was first evaluated by fluorescence microscopy and extraction, and the intracellular localization studied over time. To decipher the involved mechanisms, the transfer to membrane models (Large Unilamellar Vesicles) was then studied by fluorescence spectroscopy and discussed paying a particular attention to the dynamics of the processes.

2. Material and methods

2.1. Chemical materials

Poly(ethyleneoxide-b-ε-caprolactone) [PEO(5000)-b-PCL (4000)] (Fig. 1) were purchased from Gearing Scientific. Pheo (Fig. 1) was purchased from Frontier Scientific (Logan UT, USA). Photosensitizer stock solutions were prepared in ethanol. Experimental solutions were handled in the dark. Dioleoylphosphatidylcholine (DOPC) and L-α-phosphatidylethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Lip-Rho) were obtained from Avanti Polar Lipids (USA). 1,1′-Dioctadecyl-3,3,3,3′-tetramethylindocarbocyanine perchlorate (DiIC, Fig. 1) and 3,3′-Dioctadecyloxacarbocyanine perchlorate (DiOC, Fig. 1) fluorophores were obtained from Invitrogen Life Technologies (Saint Aubin, France). All other chemicals were purchased from Sigma (USA).

2.2. Fluorescence microscopy

The instrumental set-up was based on a Nikon Eclipse TE 300 DV inverted microscope equipped with a high-numerical aperture phase-oil objective (CFI Plan apochromat DM ×60 n.a.: 1.4, Nikon France). A 120 W metal halide lamp was used for fluorescence excitation. The mercury rays were isolated with narrow-band interference filters mounted on a filter wheel positioned along the excitation path (Sutter Instrument Company). If necessary, neutral density filters (ND×8) were used to reduce the excitation level. Image acquisition (1000 ms integration time) was performed with a CoolSNAP HQ2 (Roper Scientific France). Data acquisition and processing were performed with Metamorph software supplied by Universal Imaging Corporation (Roper Scientific, France).

2.3. Time resolved microspectrofluorimetry

Our original fluorescent confocal microscope set-up enables concomitants spectroscopic and excited state lifetime measurements of the fluorescence emission signal. A frequency domain phase-modulation method appears to be particularly appropriate for rapid and non-invasive measurements of fluorescence lifetime on single living cells. The precise description of the set-up has already been published (Petr Praus, 2007). Briefly, the 50 mW output power laser diode module (LDM 442.50.A350 from Omicron) is used for excitation at 442 nm. Modulation frequencies

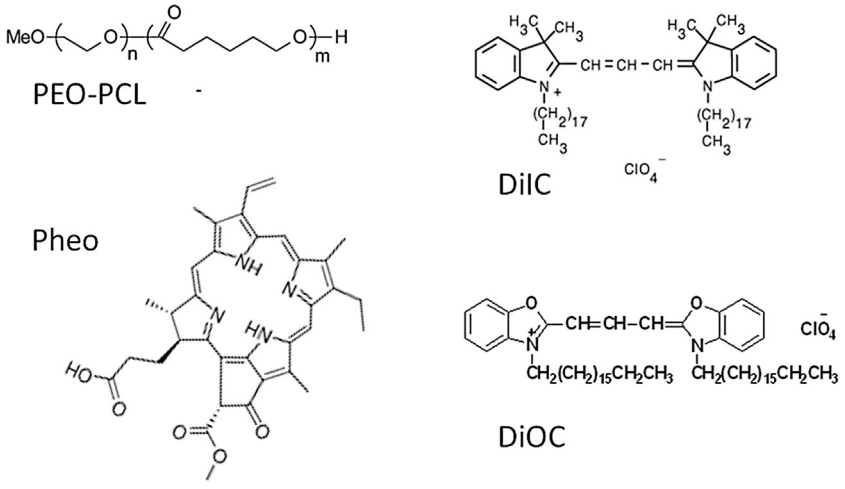

Fig. 1. Formula of the polymer (poly(ethylene oxide)-poly(ε-caprolactone) [PEO(5000)–PCL(4000)]) and of the dyes used.