Journal of Photochemistry and Photobiology A: Chemistry 314 (2016) 143–154

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Pluronic® mixed micelles as efficient nanocarriers for benzoporphyrin derivatives applied to photodynamic therapy in cancer cells

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ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form 10 August 2015
Accepted 25 August 2015
Available online 28 August 2015

Keywords:
Pluronic
Micelles
Photodynamic Therapy
Benzoporphyrin
Cancer

ABSTRACT

In this study we attempted to develop Pluronic micelles delivering the photodynamic therapy photosensitizers benzoporphyrin derivatives (BPD). The BPD A-ring (BPDMA or Verteporfin®, the active drug of FDA/USA approved Visudyne®), its regioisomer ring-B (BPDMB, not used in Visudyne® formulation due its poor solubility) and a BPDMA/BPDMB mixture (BPD-Mixt) were formulated in Pluronic P123 or F127 as well as P123/F127 mixed micelles at two different mass ratios. P123/F127 presented the lowest critical micelle concentration showing high stability due synergistic aggregation of P123 and F127. Mixed micelles allowed the encapsulation of BPD as monomers enhancing their photophysical properties and stability during time even under diluted conditions. High loading was attributed to the strong hydrophobic affinity of BPD for micelle core especially in the binary system due synergistic aggregation of P123 and F127 demonstrating the high potential of these micelles to encapsulate hydrophobic drugs. The *in vitro* assays showed a photo-activity of BPD-Mixt comparable to that of BPDMA against HeLa and A549 cancer cells under red light. The use of BPD-mixed formulations avoids the complex separation steps of these regioisomers and implies in cost reduction. The proposed system allies costs reduction and photodynamic efficiency, which stimulates further development on this nanosystem and may be of clinical interest for cancer PDT.

1. Introduction

Photodynamic therapy (PDT) is an anticancer treatment that uses a light-activated photosensitizer (PS) which interacts with molecular oxygen forming several reactive species, especially singlet oxygen ($^1O_2$), that induces cell damage as necrosis or apoptosis [1,2]. PS molecules are often characterized by low water solubility that promotes extensive self-aggregation. This inconvenient process drastically reduces PS light absorption and $^1O_2$ generation limiting PDT clinical application [2,3]. To overcome such issues, researchers have been developing several drug delivery systems to carry PS as monomers to the target sites, with subsequent increase of PS cell uptake and photodynamic activity [3].

One PS that has been very successfully applied in clinical PDT is the benzoporphyrin derivative monoacid A-ring known as BPDMA (Fig. 1). In 1999 FDA/US approved the use of BPDMA (Verteporfin®) in an aqueous liposomal formulation named as Visudyne® against age related macular degeneration [1]. The synthetic route of BPDMA naturally produces two benzoporphyrin regioisomers named as A-ring and B-ring derivatives (BPDMA and BPDMB, respectively) necessarily in equimolar quantities (Fig. 1) [4]. Despite possessing physicochemical properties and clinical activity similar to BPDMA, the B-ring derivative is not commercialized due to its high tendency to self-aggregate even in liposomal formulations [5–8]. Taking into account the high cost of BPDMA production and the high photodynamic performance of B-ring isomers coupled with the concrete possibilities for clinical treatments of tumors [9–11] it is extremely relevant to develop new drug delivery systems capable to avoid BPD self-aggregation in aqueous formulations combining selectivity and efficiency.

One promising nanomedicine-based technology for the delivery of hydrophobic molecules resides in polymeric nanoparticles, which have been widely investigated as carriers for PDT [12–15]. One representative of such materials are Pluronic® copolymers

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http://dx.doi.org/10.1016/j.jphotochem.2015.08.024
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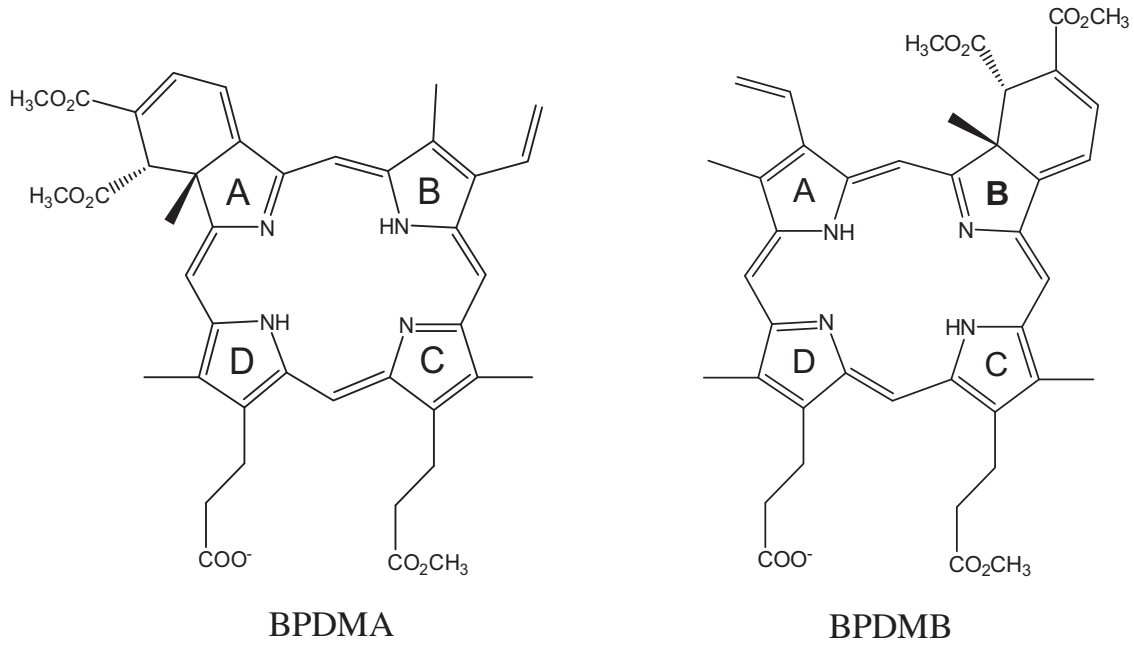

Fig. 1. Molecular structures of the benzoporphyrin derivatives monoacid A-ring (BPDMA) and ring-B (BPDMB) regioisomers.

which are surfactants molecules containing two hydrophilic poly (ethylene oxide) (PEO) and one hydrophobic poly(propylene oxide) (PPO) regions arranged in a PEO–PPO–PEO triblock structure. In water solutions and above its critical micellar concentration (CMC), Pluronic self-assembly as nanosized core–shell micelles with the PPO segment confined in the micelle core while the two PEO regions face out toward water media forming the micelle corona (shell). This nanocarrier system enables incorporation of poorly soluble drugs while increase drug circulation time and promoting passive targeting to solid tumors via enhanced permeability and retention (EPR) effect after intravenous injection [16]. Pluronic micelles have been considered a powerful and promising vehicle for the delivery of PDT agents such as porphyrins, chlorins, phthalocyanines, chlorophylls and xanthenes derivatives [6,17–19]. Moreover, previous works demonstrated that Pluronic P123 is able to solubilize B-ring benzoporphyrins as monomers in aqueous media [6,7]. Hydrophobic Pluronics such as P123 form cylindrical aggregates in aqueous medium, which exhibit higher solubilization capacity than spherical micelles formed by more hydrophilic Pluronics. However, they present low biocompatibility and stability due the excessive stacking of the cylindrical aggregates [16].

To alleviate toxicity of hydrophobic Pluronics, their combination with more hydrophilic analogs has been proposed [20] resulting in the first anti-cancer micellar formulation reaching clinical evaluation (SP1049C) [16]. The right selection of the mixture components may produce an adequate drug delivery system that manifests synergistic properties in the attempt to increase the colloidal stability and drug loading efficiency [21,22]. For example, Pluronic mixed micelles enhanced the blood circulation time of Paclitaxel increasing its cytotoxicity in multidrug resistant tumors both *in vitro* and *in vivo* as compared to Taxol® [23,24]. A blend of hydrophobic P105 and hydrophilic Pluronic F127 incorporating Docetaxel was developed for the treatment of lung multidrug resistance tumors [22]. Recently, Pepić et al. developed a L121/F127 Pluronic mixed micelles that demonstrated a high colloidal stability upon dilution in biological relevant media [25].

To increase stability and biocompatibility of P123 micelles here we propose a mixed micelle system with the hydrophilic F127. The choice of Pluronic F127 aims to increase PEO length at micelle surface, which should make the micelle more prone to long-circulation time while improving biocompatibility [16]. Additionally, PPO segment in P123 and F127 have the same length, which is highly desirable to enhance micelle stability [23]. Micelles were employed to formulate BPDMA, BPDMB and their 1:1 mixture (BPD–Mixt) in view of applications in photodynamic therapy. The use of B-ring derivative itself or mixed with A-ring derivatives aims costs reduction, once a total solubilization and stabilization of the compounds in the monomeric photoactive form is achieved. To this purpose, BPD-Pluronic interactions were studied and photophysical/physicochemical properties, *in vitro* release behavior as well as stability of BPD-loaded micelles were evaluated. In addition, photo-activity of the BPD formulation was investigated by *in vitro* assays on HeLa and A549 cancer cell lines.

2. Materials and methods

2.1. Materials

BPDMA and BPDMB (MW = 718.8 g mol^{-1}) were kindly supplied by Professor D. Dolphin (University of British Columbia, Vancouver, Canada). Pluronics P123 (EO_{20}–PO_{65}–EO_{20}, MW = 5750 g mol^{-1}) and F127 (EO_{100}–PO_{65}–EO_{100}, MW = 12,600 g mol^{-1}) were purchased from Sigma–Aldrich and the solutions were prepared by weighting materials previously dried under vacuum for 24 h. Trehalose, pyrene and 1,6 diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma–Aldrich. MitoTracker® Green FM, *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diazas-indacene-3-pentanoyl) sphingosine (BODIPY® FL C5-ceramide), LysoTracker® Green DND-26, and ER-Tracker™ Green (glibenclamide BODIPY®FL), were purchased from Molecular Probes (Milan, Italy). The CellTiter96® Aqueous one solution cell proliferation Assay (MTS) was from Promega Co. (Madison, USA). All the other chemicals were of analytical reagent grade and used without previous purification.