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Tumour-localizing and -photosensitising properties of *meso*-tetra(4-nido-carboranylphenyl)porphyrin (H_2TCP)

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Abstract

A water-soluble *meso*-substituted porphyrin (H_2TCP) bearing 36 boron atoms, which appeared to be an efficient photodynamic sensitiser (singlet oxygen quantum yield = 0.44), was studied for its accumulation by murine melanotic melanoma cells (B16F1). The amount of H_2TCP in the cells increased with the porphyrin dose in the incubation medium up to, and at least, 100 μM concentrations with no significant cytotoxic effect in the dark. Moreover, the H_2TCP uptake increased with the incubation time reaching a plateau value corresponding with the recovery of 0.4 nmol of H_2TCP per mg of cell proteins after 24 h incubation. Fluorescence microscopy observations showed that the porphyrin was largely localized intracellularly, exhibiting a discrete distribution in the cytoplasm with a pattern which was closely similar to that observed for the endosomal probe Lucifer yellow. The photosensitising efficiency of the H_2TCP toward B16F1 cells was studied for different irradiation (1–15 min) and incubation (1–24 h) times. Nearly complete (>95%) cell mortality was obtained upon incubation with 20 μM H_2TCP and 10 min irradiation with red light (600–700 nm, 20 mW/cm^2). The porphyrin was also accumulated in appreciable amounts by the tumour tissue after intravenous injection to C57BL/6 mice bearing a subcutaneously transplanted melanotic melanoma. Maximum accumulation in the tumour was achieved by administration of H_2TCP dissolved in the ternary mixture 20% dimethylsulfoxide (DMSO)–30% polyethyleneglycol (PEG 400)–50% water. Thus, this porphyrin could act as both a photodynamic therapy agent and a radiosensitising agent for boron neutron capture therapy.

Keywords: Photodynamic therapy; Boron neutron capture therapy; Porphyrin; Carborane; Melanotic melanoma

1. Introduction

Among the modalities for tumour treatment which are currently under active investigation in view of palliative or curative applications, boron neutron capture therapy (BNCT) and photodynamic therapy (PDT) represent two emerging therapeutic strategies [1,2]. Both modalities involve the systemic injection of an intrinsically non toxic radio- or photo-sensitising agent to the patient, followed by the specific irradiation of the diseased area with thermal neutrons in the case of BNCT [3,4] or selected visible light wavelengths for PDT [5]. In particular, BNCT represents a binary modality for cancer treatment that involves the irradiation of ^{10}B-enriched tumour lesions with low energy (thermal, ~0.025 eV) neutrons, resulting in the release of highly cytotoxic particles, $^4He^{2+}$ (α-particle of 1.42 MeV of energy) and $^7Li^{3+}$ ions of 0.84 MeV of energy. These ions are capable of causing severe damage to biological

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materials; the toxic effect is most likely confined within the ^{10}B-containing cell since such ions display limited paths of travel in tissue (9–5 μm, respectively, i.e., about one cell diameter) [1,3,4].

On the other hand, PDT is based on the combined action of visible photons with photosensitising agents and molecular oxygen leading to a sequence of photochemical and photobiological reactions that result in irreversible damage to the tumour. During this process the electronic energy initially absorbed by the photosensitiser is transferred to ground-state oxygen to produce singlet oxygen ($^1O_2$) and oxygen radicals, which further react with cellular components to cause cell death [6]. At present, regulatory approval is limited to borono-phenylalanine and sodium borocaptate [7,4] for BNCT, as well as to Photofrin, a complex mixture of haematoporphyrin derivatives, and 5-aminolevulinic acid (ALA), a metabolic precursor of protoporphyrin IX, for PDT [8]. A large number of patients have been treated by either technique worldwide with objectively positive results. However, for both techniques, severe limitations exist owing to the relatively low selectivity of tumour targeting by the above mentioned radio- or photo-sensitising agents coupled with the small efficiency of their interaction with the incident radiation [3,5]. Recently, new perspectives have been opened in this field by the synthesis of chemically pure boron-labelled porphyrins and phthalocyanines [9], where the tetrapyrrolic derivative can act as a vehicle for the transport of significant amounts of boron to the neoplastic lesion; at the same time, many newly developed porphyrins and phthalocyanines have been shown to possess satisfactory tumour-photosensitising properties which are markedly superior to those typical of Photofrin [10]. In principle, such boron-loaded porphyrins/phthalocyanines could allow the development of combination therapies based on the sequential application of BNCT and PDT, which have the possibility to act synergistically given the different mechanisms of action of the two therapeutic approaches.

In this paper, we describe our findings on the affinity for tumour cells in vitro and for tumour tissues in vivo of a water-soluble *meso*-substituted tetra(nido-carboranylphenyl)porphyrin (H_2TCP) carrying 36 boron atoms per molecule, which should thus exhibit a large cross section for interaction with neutrons [11]. The effect of the large boron clusters on the photodynamic activity of this porphyrin was also tested. The chemical structure of H_2TCP is shown in Fig. 1.

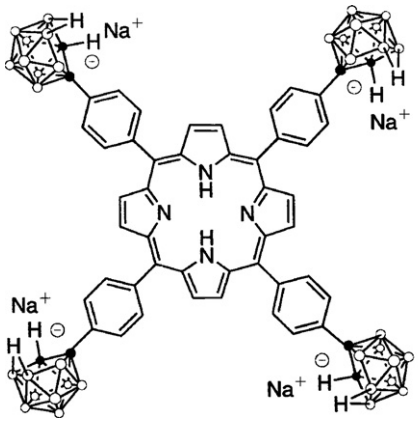

Fig. 1. Chemical structure of H_2TCP.

2. Materials and methods

2.1. Porphyrin synthesis

H_2TCP [*meso*-tetra(4-nido-carboranylphenyl)porphyrin] was prepared by chemical synthesis in the Department of Chemistry at Louisiana State University in Baton Rouge, USA following a synthetic route similar to that previously published [11]. Our optimized synthesis of H_2TCP uses $BF_3 \cdot OEt_2$ (0.1 mmol) as the acid catalyst in the condensation of 4-carboranylbenzaldehyde (1 mmol) with pyrrole (1 mmol); under these conditions H_2TCP was obtained in 53.9% yield after purification by column chromatography on silica gel using dichloromethane/hexane 1:2 for elution, and recrystallization from dichloromethane/methanol. The chemical structure of H_2TCP was characterized by standard spectroscopic and chemical analytical techniques.

2.2. Determination of singlet oxygen quantum yield

The quantum yield (Φ_Δ) of singlet oxygen generation by H_2TCP was measured by following the decrease in the fluorescence emission of 9,10-dimethyl-anthracene (DMA) upon its photosensitised conversion into the corresponding non-fluorescent 9,10-endoperoxide [12]. In a typical experiment, a 20 μM DMA and 1.4 μM H_2TCP solution in methanol was placed in a quartz cuvette of 1 cm optical path and irradiated for different periods of time at 20 ± 2 °C under gentle magnetic stirring by 600–700 nm light. This wavelength interval was isolated from the emission of a halogen lamp by the insertion of broadband optical filters (Waldmann, Schwenningen, Germany). The fluence-rate was 100 mW/cm^2. The DMA fluorescence emission was recorded in the 380–550 nm wavelength range with excitation at 360 nm. The first-order rate constant of the photoprocess was obtained by plotting $\ln F_0/F$ as a function of the irradiation time, where F_0 and F represent the fluorescence intensity at time 0 and at time t, respectively. The rate constant was then converted into $^1O_2$ quantum yield by comparison with the rate constant for DMA photooxidation sensitized by haematoporphyrin (Hp) for which Φ_Δ was shown to be 0.65.

2.3. Fluorescence quantum yield

The fluorescence quantum yield for H_2TCP in methanol was measured by using a Perkin–Elmer LS 50 spectrophotofluorimeter. *meso*-Tetrakis(*p*-sulfonatophenyl) porphyrin (TPPS) in neutral aqueous solution was used as a reference