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Photodynamic effects of novel 5,15-diaryl-tetrapyrrole derivatives on human colon carcinoma cells

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ABSTRACT

Preliminary in vitro cytotoxicity studies on a panel of *meso* diaryl-substituted tetrapyrrole derivatives newly synthesized in our laboratory have shown that these compounds are photodynamically active on the human colon carcinoma cell line HCT116. In the present study, we investigate some mechanistic aspects of the photodynamic action of the most active compounds in the series, namely the 5-phenyl-15-(3-methoxyphenyl)porphyrin (**1**), the 5-phenyl-15-(3-hydroxyphenyl)porphyrin (**2**) and the 5,15-diphenylporphyrin (**3**). The results of the cytotoxicity studies indicate that the novel photosensitisers (PSs) are more potent in vitro than *m*-THPC (Foscan®), a powerful PS already approved for clinical use in photodynamic therapy (PDT). A series of experiments were performed to elucidate a number of aspects in the mechanism of PS-induced phototoxicity, including, intracellular accumulation and subcellular localization of the PSs, induction of apoptosis, and generation of reactive oxygen species (ROS) and NO·. All the compounds tested exhibit similar singlet oxygen quantum yields; differential intracellular accumulation can contribute to the observed differences in phototoxicity. Flow cytometric studies indicate that all the tested compounds induce apoptosis; however, their cytotoxic effect does not seem to rely solely on this process. Generation of significant amounts of reactive oxygen species (ROS) and NO· were also observed; however, the contribution of this latter effect to the overall phototoxicity is unclear. Taken together, our observations suggest that the diaryl derivatives included in the present study could represent promising leads for the development of novel photosensitizing agents.

1. Introduction

Photodynamic therapy (PDT) is a minimally invasive treatment for both neoplastic and non-neoplastic proliferating cell diseases.[1,2] PDT relies on the uptake of a photosensitizing compound by the pathologic tissue. Subsequent activation of the PS by local irradiation with visible light results in singlet oxygen production, which is followed by localized cellular damage. Tumor ablation is thought to depend both on direct lethal effects of PDT on tumor cells and on microvascular injury induced by the treatment, thus limiting tumor blood supply.[2–4] Cell death was initially thought to occur by necrosis; however, apoptosis and, more recently, autophagy have also been implicated in the process, depending on the PS used and its concentration, light dose, oxygen availability and the cell type(s) involved.[5–9]

The first PS to be granted regulatory approval (in 1993) was porfimer sodium (Photofrin®, Axcan Pharma, Montreal, Canada), a mixture of porphyrin oligomers produced by partial purification of hematoporphyrin derivative. Photofrin®-based PDT has proven effective against a variety of tumors, including cancers of the lung, stomach, cervix, bladder and esophagus. However, its weak absorption in the red region of the spectrum ($\geqslant$600 nm, necessary for the PS activation in the deeper tissues with a penetrating red light) and the extended skin photosensitivity make this PS less than ideal for clinical use, thus prompting an active search for novel PSs featuring more favorable properties.[10] Various classes of PSs are currently in clinical use or at different stages of preclinical and clinical development, many of which include the cyclic tetrapyrrole core structure typical of porphyrins (for a recent review see[11]). Among these 'second generation' PSs, the synthetic chlorin derivative 5,10,15,20-tetra(3-hydroxyphenyl)chlorin (*m*-THPC, also known as temoporfin; marketed as Foscan® by Biolitec Pharma, Scotland, UK) is perhaps the most successful, having recently been granted European approval for palliative treatment of patients with advanced head and neck cancers.[12,13] Encouraging preclinical in vitro and in vivo

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results have been reported by Patrice and co-workers,[14] using a diaryl-substituted porphyrin derivative. More recently, our group has synthesized a series of novel 5,15-diaryl-tetrapyrrole derivatives, most of which show greater phototoxicity than either Photofrin® or *m*-THPC in a human colon cancer cell line in a preliminary in vitro screening.[15]

In the present study, we focus on the most active compounds from this preliminary screen (Fig. 1), and we assess some mechanistic aspects of their photodynamic activity in the human colon carcinoma cell line HCT116. Thus, a series of experiments have been performed in vitro with photosensitizers **1**, **2**, and **3,** using *m*-THPC as reference compound and a 500 W tungsten–halogen white lamp to irradiate the cells. Besides the dose–response curves, the effects of the PSs on the following issues were investigated: (a) intracellular generation of oxidizing species, including singlet oxygen as well as other reactive oxygen species (ROS) and with a special emphasis on NO·; (b) intracellular accumulation and subcellular distribution of the PSs; (c) involvement of apoptosis in PS-induced cell death; (d) cell cycle distribution; (e) expression of a small panel of proteins involved in apoptosis control. The results reported here confirm what we have recently published[15], namely that the novel diaryl-substituted porphyrins are generally more active, at least in vitro, than *m*-THPC. These observations suggest that porphyrins in this class are promising candidates for further transformation into the corresponding chlorin derivatives, yielding potent PSs suitable for in vivo photodynamic therapy. This is confirmed by the superior phototoxicity exhibited by the chlorin derivative obtained from compound **2** as compared with *m*-THCP.

2. Results

2.1. Phototoxicity studies and Western blot analysis

Table 1 reports the IC_{50} values extrapolated from the dose–response curves obtained with HCT116 cells following PDT with the four PSs (Fig. 1), irradiating with white light. Intrinsic cytotoxicity of the PSs, assessed by omitting the irradiation step from the treatment protocol, was found to be negligible in all cases, up to PS concentrations 10-fold higher than those used for PDT experiments (not shown). Overall, the newly synthesized derivatives were more phototoxic than *m*-THPC, even though statistically significant differences in potency were only achieved by compound **2,** versus both *m*-THPC and the other two diaryl derivatives **1** and **3**.

Table 1
IC_{50} values obtained with HCT116 cells following 24 h exposure to the PSs, 2 h irradiation in PS-free PBS and 24 h incubation in PS-free complete medium

	IC_{50} (nM)
m-THPC	7.6 ± 0.57
1	5.94 ± 0.85
2	1.06 ± 0.15*
3	5.66 ± 1.16

Mean ± SE of 3–4 independent experiments.
* $p < 0.05$ versus all other PSs.

Figure 2A shows apoptosis induction following 24 h exposure to equitoxic concentrations (IC_{50}) of the PSs, followed by 2 h irradiation and 24 h incubation in PS-free medium. All the PSs tested were able to induce apoptosis to some extent, even though the increase in apoptotic cells over control samples only attained statistical significance in the case of *m*-THPC; no significant differences could be observed among all treated groups. Western blot analysis of total protein extracts from treated cells indicates a decrease in the expression of antiapoptotic proteins, such as Bcl2 and survivin, and an increase in the proapoptotic factor Bax, as compared to control cells (Fig. 2B). Such alterations are more marked in extracts from *m*-THPC-treated cells, supporting the stronger proapoptotic activity exhibited by this compound. Cell cycle analysis did not show any significant modifications in cell distribution (Table 2); more specifically, treated cells do not appear to arrest in the G1 or G2/M phases of the cell cycle.

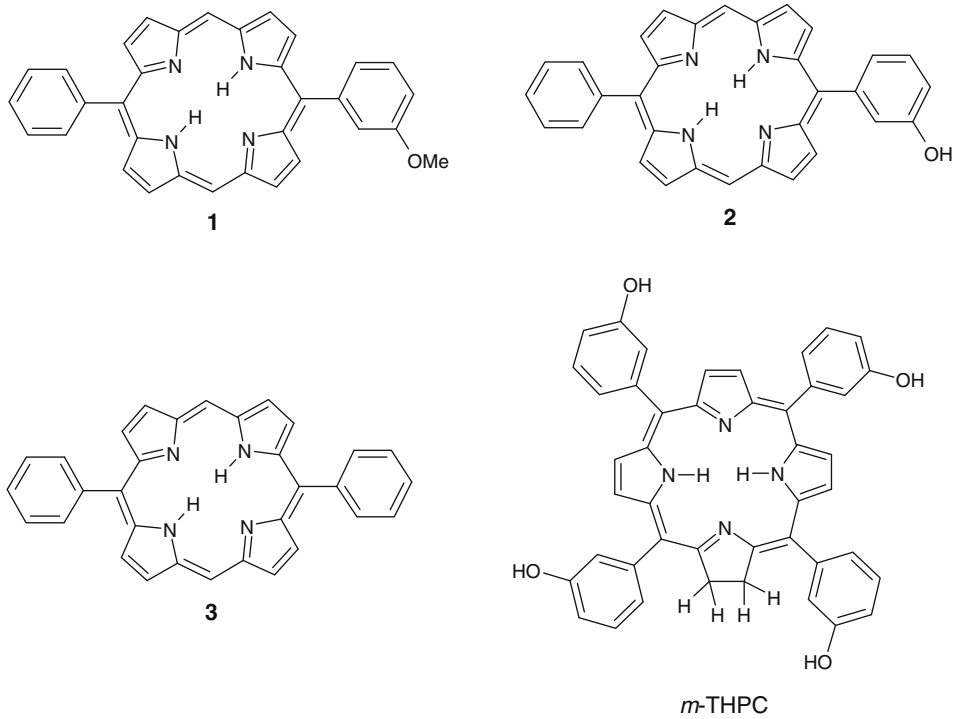

Figure 1. Chemical structures of the 5,15-diarylporphyrins (**1**–**3**) and of *m*-THPC.