British Journal of Pharmacology (2008) 154, 1–3

0007–1188/08 $30.00
www.brjpharmacol.org

COMMENTARY

Photodynamic therapy: novel third-generation photosensitizers one step closer?

LB Josefsen and RW Boyle

Department of Chemistry, University of Hull, Kingston-upon-Hull, UK

Photodynamic sensitizers are drugs activated by light of a specific wavelength and are used in the photodynamic therapy (PDT) of certain diseases. Second- and third-generation photosensitizers with improved PDT properties are now under investigation. In this issue of the *British Journal of Pharmacology*, Leung *et al*. have described the synthesis and investigation of a second-generation photosensitizer (BAM-SiPc) targeted towards the cells of HepG2 and HT29 tumours. BAM-SiPc is selectively functionalized with bis-amino groups and has demonstrated potent PDT activity in a small animal model. However, it also exhibited non-selective distribution and accumulation in multiple animal (small mouse) organs and tissue. These issues highlight the importance and need for good biodistribution and localization properties for an efficacious photosensitizer. The lack of tumour specificity may have a significant impact on the potential BAM-SiPc has in clinical PDT.
British Journal of Pharmacology (2008) **154,** 1–3; doi:10.1038/bjp.2008.98; published online 24 March 2008

Keywords: biodistribution; photodynamic therapy; photosensitizers; phthalocyanine; targeting

Abbreviations: LDL, low-density lipoproteins; Nc, naphthalocyanine; PDT, photodynamic therapy

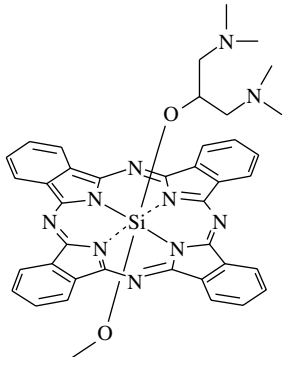
Introduction

Photodynamic sensitizers are molecules that, when activated by light of a specific wavelength and in the presence of molecular oxygen, generate reactive oxygen species. In biological systems, these cytotoxic species trigger a cascade of biochemical responses that result in cell death. The observed photodynamic effect is local rather than systemic in nature as the light is applied specifically at the treatment site (Brown *et al*., 2004).

Clinically, photodynamic therapy (PDT) makes use of the photodynamic effect to bring about the selective destruction of cancerous and non-neoplastic tissues (MacDonald and Dougherty, 2001). PDT is gradually becoming a more widely used medical tool and has received regulatory approval for the treatment of a number of diseases worldwide, particularly age-related macular degeneration and certain solid tumours (MacDonald and Dougherty, 2001; Brown *et al*., 2004). However, the use of PDT is frequently limited by associated side effects, such as nonspecific biodistribution and prolonged accumulation in non-neoplastic tissue (MacDonald and Dougherty, 2001).

First-generation PDT sensitizers, such as Photofrin®, exhibited prolonged patient photosensitivity (poor clearance) and lacked long wavelength absorption (MacDonald and Dougherty, 2001): important factors contributing to the limitation of these photosensitizers in PDT. The synthesis of improved (second-generation) photosensitizers moved towards modified tetrapyrrolic (porphyrin®) compounds, such as benzoporphyrin (Visudyne®), chlorin (Temoporfin®) and porphycene (ATMPn), which have a more intense long wavelength absorption (MacDonald and Dougherty, 2001; Brown *et al.,* 2004). Metallated derivatives have also been synthesized (Al, $AlPcS_4$; Si, SiNC (Nc—naphthalocyanine); and Sn, $SnEt_2$; Ali and van Lier, 1999) and investigated, although there is no consistent correlation between metallation and increased photodynamic activity (Josefsen and Boyle, 2008). More recently, targeting strategies have been shown to increase the affinity of the photosensitizer for tumour tissue (Hudson *et al.,* 2005). There have also been reports of selectively targeting subcellular compartments, including the mitochondria (Dummin *et al.,* 1997). These targeting approaches have led to third-generation photosensitizers and some of the most promising results to date.

In this issue of the *British Journal of Pharmacology*, Leung *et al.* (2008) describe a potent second-generation photo-



Correspondence: Dr RW Boyle, Department of Chemistry, University of Hull, Kingston-upon-Hull HU6 7RX, UK.
E-mail: r.w.boyle@hull.ac.uk
Received 13 February 2008; accepted 27 February 2008; published online 24 March 2008

sensitizer, bis-amino silicon(IV) phthalocyanine (BAM-SiPc). The authors synthesized and investigated the amphiphilic SiPc in a range of different cancer cell lines and in tumour-bearing nude mice. Leung *et al.* (2008) report that BAM-SiPc has a high phototoxicity against HepG2 tumour tissue and significantly retards the growth of HT29 tumour cells in the mouse model. They also observed apoptotic processes in the (PDT) treatment of the HepG2 tumour. However, the authors also report that the biodistribution of the BAM-SiPc photosensitizer is nonspecific in a small animal model. They observed a significant accumulation of BAM-SiPc in the liver, lungs, spleen, kidneys, muscle and skin tissue of the mice. Accumulation of the photosensitizer in the lungs, liver, spleen and kidneys was almost 40–70% greater than in the tumour tissue itself. However, the BAM-SiPc photosensitizer did clear more rapidly from the liver (within 1 week) and have minimal dark toxicity in comparison to other approved second-generation photosensitizers (Leung *et al.,* 2008)—promising properties with respect to clinical PDT.

The paper by Leung *et al.* (2008) highlights the need for increased selectivity of photosensitizers for tumour tissue over healthy tissue—without this specificity PDT has limited clinical applications. This problem can be addressed by increasing the affinity of the photosensitizer for the tumour tissue by targeting the photosensitizer specifically towards the tumour site/cells. Conjugating a targeting component, such as an antibody (directed against the tumour antigens), towards the photosensitizer allows the drug to localize, accumulate and bind selectively at the diseased site (Konan *et al.,* 2002; Hudson *et al.,* 2005; Staneloudi *et al.,* 2007). The photosensitizer bioconjugate is then able to (specifically) photodynamically inactivate in tumour cells expressing the tumour-associated antigen, minimizing healthy cell localization and concomitant damage. Other receptor-positive sites on the tumour surface, such as LDLs (low-density lipoproteins) and folate receptors could be taken advantage of by conjugating the photosensitizer to LDL or folate molecules (Konan *et al.,* 2002): LDL and folate receptors are overexpressed on tumour cell surfaces. An alternative approach would be to use a molecular carrier such as a liposome or targeted nanospecies (Konan *et al.,* 2002; Castano *et al.,* 2005). Attaching targeting components to the photosensitizer also has an additional benefit. A number of photosensitizers that have shown promise *in vivo* exhibit poor solubility in aqueous media, preventing intravenous delivery into the bloodstream and affecting their efficacy and use in physiological media and the clinic.

Investigations into third-generation photosensitizers bearing targeting moieties (Konan *et al.,* 2002; Hudson *et al.,* 2005; Staneloudi *et al.,* 2007) have demonstrated that photosensitizers directed against tumour tissue have minimal (photosensitizer) accumulation in normal tissue, while a high tumour binding specificity is observed. Furthermore, Staneloudi *et al.* (2007) found that photosensitizers conjugated to a single-chain monoclonal antibody (scFv) fragment are also more effectively cleared from the circulation than photosensitizer–monoclonal antibody conjugates alone.

From the study conducted by Leung *et al.* (2008), the following questions are raised: 'what determinants cause the photosensitizers to accumulate at certain sites and can PDT progress/succeed any further without the synthesis and approval of highly selective photosensitizers directed specifically against diseased tissue?'

The chemical structure of a photosensitizer plays a key role in the success of the compound as a PDT agent. Photosensitizers need to be soluble in physiological media: the degree of photosensitizer hydrophilicity and amphiphilicity directly affects its route of administration and the biodistribution/pharmacokinetic profile (MacDonald and Dougherty, 2001; Castano *et al.,* 2005). Photosensitizers bearing certain structural characteristics have been reported to localize selectively in tumour tissue. Although the localization mechanisms are not fully understood, the more hydrophobic photosensitizers have demonstrated tumour to normal tissue ratios of 7:1 and 8:1, whereas the equivalent hydrophilic photosensitizers exhibited a 2:1 ratio (Ruck and Steiner, 1998). Photosensitizers with anionic substituents, such as sulphonate or carboxyl groups, have been observed to localize preferentially in the cytoplasm and relocate to the nucleus upon illumination (Patito *et al.,* 2001), whereas lipophilic photosensitizers functionalized with cationic groups are believed to (preferentially) traverse the mitochondrial membrane and accumulate in the mitochondrion (Dummin *et al.,* 1997) — the subcellular organelle widely demonstrated to be a key component in the preferred (apoptotic) cell death pathway. Exactly which physicochemical/structural properties and mechanisms are behind these specific distributions and localizations and how to maximize tumour tissue selectivity over normal tissue accumulation are issues still under investigation.

Leung *et al.* (2008) demonstrated that BAM-SiPc is an effective PDT photosensitizer in (certain) cancer cell lines and provide information (from a small animal model) indicating that BAM-SiPc is a potentially potent photosensitizer that effects tumour cell death through the more favourable apoptotic pathway. However, there are biodistribution and localization problems associated with this photosensitizer. The nonspecific distribution and localization of BAM-SiPc may limit its use and efficacy in clinical PDT. The PDT effect observed from BAM-SiPc highlights the importance and need for tumour-specific photosensitizer targeting and minimal accumulation of the photosensitizer in non-tumour tissue, either through selective activation of the photosensitizer with substituents, by cellular function-sensitive linkages or via conjugation to macromolecules/biomolecules. The results of the study by Leung *et al.* (2008) suggests that BAM-SiPc photosensitizers show potential for use as a PDT agent. Clearly, the development of novel photosensitizers with tumour-specific properties will lead to more effective PDT and new applications for these drugs.

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